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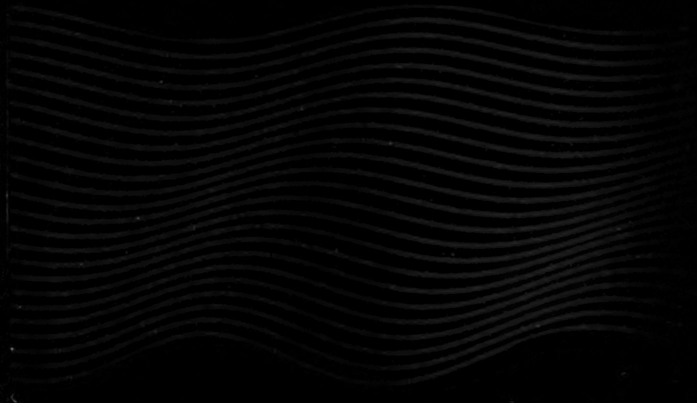

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Predation by sea lamprey (*Petromyzon marinus*) on lake trout (*Salvelinus namaycush*) in southern Lake Ontario, 1982–1992[1,2]

C.P. Schneider, R.W. Owens, R.A. Bergstedt, and R. O'Gorman

Abstract: Dead lake trout (*Salvelinus namaycush*) killed by sea lamprey (*Petromyzon marinus*) were collected from the bottom of Lake Ontario using bottom trawls. The number of dead lake trout per hectare could be predicted from the number of type A-1 sea lamprey marks observed on live fish in September gillnet surveys ($r^2 = 0.60$, $P < 0.01$) but not from the sum of marks of types A-1, A-2, and A-3 combined. Sea lamprey selectively attacked and killed the largest lake trout. The lengths and ages of live fish with A-1 marks increased as the population of longer, older lake trout in the lake increased, and the length distributions of fish killed by sea lamprey were not different ($P > 0.05$) from those of live fish with A-1 marks in 5 of 6 years where comparisons could be made. Compared with Lake Superior strain lake trout, Seneca Lake strain fish were only 0.41 times as likely to be attacked by sea lamprey and were less likely to die from an attack (both differences $P < 0.05$). Conservative estimates of the numbers of lake trout killed by sea lamprey in southern Lake Ontario from October to mid-November ranged from 17 000 in 1988 to 121 000 in 1984.

Résumé : Au moyen d'un chalut de fond, nous avons prélevé au fond du lac Ontario des touladis (*Salvelinus namaycush*) qui avaient été tués par des lamproies marines (*Petromyzon marinus*). Le nombre de ces poissons morts par hectare pouvait être prédit à partir du nombre de cicatrices de lamproie de type A-1 observées sur les poissons vivants dans le cadre de relevés effectués en septembre au moyen de filets maillants ($r^2 = 0{,}60$, $P < 0{,}01$), mais non à partir du nombre total des cicatrices A-1, A-2 et A-3. La lamproie marine attaque et tue de manière sélective les touladis les plus gros. La longueur et l'âge des poissons vivants portant des cicatrices A-1 augmentait à mesure que les touladis plus vieux et plus longs se faisaient plus nombreux dans le lac, et la distribution des tailles chez les poissons tués par les lamproies n'était pas différente ($P > 0{,}05$) de celle observée chez les poissons vivants portant des cicatrices A-1, pour 5 des 6 années où une telle comparaison était possible. La probabilité qu'un touladi de la souche du lac Seneca soit attaqué par une lamproie équivalait à seulement 0,41 fois la probabilité qu'un touladi de la souche du lac Supérieur subisse le même sort, et les attaques étaient moins souvent mortelles dans le premier cas ($P < 0{,}05$ pour les deux différences). Les estimations prudentes du nombre de touladis tués par les lamproies marines dans le sud du lac Ontario entre octobre et la mi-novembre vont de 17 000 en 1988 à 121 000 en 1984.
[Traduit par la Rédaction]

Introduction

When the parasitic sea lamprey (*Petromyzon marinus*) invaded the Great Lakes it caused extensive changes in the fish communities (Smith 1968; Christie 1974). Sea lamprey preyed heavily on lake trout (*Salvelinus namaycush*), the Great Lakes' main piscivore, eliminating them from much of their historic range during the 1940s and 1950s (Hile et al. 1951; Eschmeyer 1957; Coble et al. 1990; but see Eshenroder et al. 1992). Sharply reduced predation allowed prey species to proliferate, particularly the exotic alewife (*Alosa pseudoharengus*). Development of techniques in the late 1950s to control the sea lamprey (Smith and Tibbles 1980; Pearce et al. 1980) made it feasible to reintroduce lake trout throughout the Great Lakes. Following control of sea lamprey in Lake Ontario, about 1 million lake trout were stocked annually in U.S. waters during 1980–1992 (Elrod et al. 1995). But despite reductions in the number of sea lamprey, they still kill substantial numbers of lake trout in some areas and thus are impeding the rebuilding of depleted populations (Pycha and King 1975; Pycha 1980; Bergstedt and Schneider 1988).

Information gained from collections of lake trout killed by sea lamprey can be used to improve our understanding of sea lamprey attack lethality and vulnerability of lake trout strains to attack. Direct estimates of the density (number per hectare) of dead lake trout can be used to validate damage estimates and goals for sea lamprey control. Although the sea lamprey remains a potent source of lake trout mortality, there is still ambiguity about selection of prey by sea lamprey because investigators could only examine the survivors of sea lamprey

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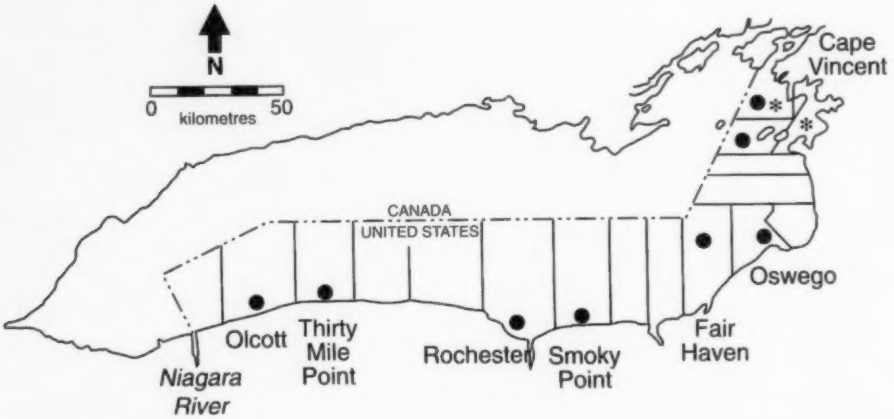
Fig. 1. Lake Ontario showing 17 areas in which gillnets were fished during September 1982–1992. Asterisks show two areas where gillnet transects were fixed rather than randomly located. Dots show the eight areas in which dead lake trout were recovered with bottom trawls during October–November 1982–1992.



attacks (fish bearing marks from attacks) and not the victims (dead fish). For example, collections of lake trout from Lake Michigan during their decline in the late 1940s showed the proportion with a sea lamprey mark increasing from less than 1% among fish 127–429 mm to 80% among fish 622–899 mm (Van Oosten 1949). This typical increase in marking rate with size of lake trout could be due either to higher lethality of attacks on smaller hosts (Eschmeyer 1957) or to selection of larger fish as hosts (Fry and Budd 1958; Pycha and King 1975). If sea lamprey select the largest lake trout, then rebuilding spawning populations will be hampered. In contrast, rebuilding stocks of extirpated lake trout could proceed faster if hatcheries produced strains of lake trout that were less likely to be attacked or were less likely to succumb to an attack. Although resistance to sea lamprey has been hypothesized (Christie 1973), documentation from field studies is lacking, most likely because results would have been confounded without the ability to examine dead lake trout.

To examine prey selection and lethality of attacks by sea lamprey, we collected live lake trout with gillnets and dead lake trout with bottom trawls in the U.S waters of Lake Ontario during 1982–1992. To examine prey selection, we compared length, age, sex, and strain of live lake trout with those of lake trout killed by sea lamprey. The lethality of sea lamprey attacks among the various sizes and strains of lake trout was assessed by comparing numbers of live lake trout marked by sea lamprey with lake trout killed by sea lamprey. We also examined which classification of sea lamprey marks (King 1980) best reflects the number of fish killed. Finally, to estimate sea lamprey predation, we updated Bergstedt and Schneider's (1988) estimates of the number of lake trout killed in U.S. waters of Lake Ontario in light of new information on area swept by the trawls.

Methods

Live lake trout

Gillnets were fished at transects within each of 17 areas in U.S. waters of Lake Ontario during September 1982–1992 (Fig. 1). The transects were perpendicular to shore and, within 15 of the areas, were randomly located along a minute of longitude (south shore areas) or a minute of latitude (east shore areas). Because of limited area deep enough for lake trout to inhabit and limited flexibility for us to randomize sampling location, fixed transects were fished each year in the two northeasternmost areas.

The multifilament gillnets were made up of nine 15.2 × 2.4 m panels of mesh sizes ranging from 51 to 151 mm (stretched measure) by 12.5-mm increments and were fished overnight along the contour. Four nets were usually fished at each transect, with the first net set at a depth where the 10–12°C isotherms intersected the bottom. In 1980–1983 the second and third nets were usually set 5 m successively deeper than the first net, and the fourth net was set 10 m deeper than the third. In 1984–1992 the second, third, and fourth nets were each set 10 m successively deeper. In the northeastern areas where depths were limited, all four nets could not be fished in years when the thermocline was deep. Our measure of annual lake trout abundance was the total catch in the gillnets unadjusted for effort because the differences among years in the number of nets fished and their placement was small (range 58–68 nets), with differences mostly confined to the deeper sets, which caught the fewest fish.

Recovering dead lake trout

Bottom trawls were towed along and across depth contours, during October 1 – November 15 to determine the density of dead lake trout on the lake floor. Dead lake trout were recovered at other times of the years, although nearly all lake trout killed by sea lamprey were collected in autumn (Bergstedt and Schneider 1988; Elrod et al. 1995). Recovery methods during 1986–1992 were similar to those described by Bergstedt and Schneider (1988). Trawl tows along the contour were part of an annual assessment of live prey fish and were mostly of 10-min duration; trawl tows across contours were solely to recover dead lake trout and were of longer duration (up to 120 min). Along-contour tows were always made with a 12-m, bottom trawl. Across-contour tows began in 1983 and were made only with the 12-m trawl until 1985. Thereafter, the 12-m trawl was used in combination with bottom trawls with headropes of either 18 m (1985) or 20 m (1986–1992). During along-contour tows, the trawl was fitted with a cod end of 9-mm mesh (stretched measure) whereas during the longer, across-contour tows the trawl was fitted with a cod end of 89-mm mesh (stretched measure).

In most years we trawled along and across contours for dead lake trout in six areas: Olcott, Thirty Mile Point, Rochester, Smoky Point, Fair Haven, and Oswego (Fig. 1). In 1983, along-contour trawling was restricted to the Fair Haven and Oswego areas. After 1984, we also made across-contour tows in two areas near Cape Vincent.

Area swept by the trawls was calculated from the linear distance the net was towed and the distance between the wing tips of the trawl (net spread). Measurements were made with SCANMAR[3] acoustic units attached to the wing tips. The net spread was 9.5 m for the 12-m trawl and 12.8 m for the 20-m trawl. Heretofore, the spread of the 12-m trawl was reported as 6.4 m based on measurements made by scuba divers in the mid-1970's (Bergstedt and Schneider 1988); resulting in lower estimates of area swept. The spread of the 18-m trawl was 14 m when measured with dacron line and fishing reels attached to the wing tips.

We calculated the number of dead lake trout on bottom by multiplying the density of dead lake trout bearing fresh sea lamprey marks in each of three depth strata (30–100, 100–160, and ≥160 m) by the total number of hectares in each stratum. Of the bottom area swept each year, most was in the 30- to 100-m stratum and the remainder of the bottom area swept was about equally divided between two deeper strata (Table 1). For 1982–1984, when all three strata were not sampled, density of dead lake trout in the strata that were not sampled was estimated from the density in the adjacent sampled strata by the mean ratio of densities among strata in years when all three depth strata were sampled. Small numbers of dead lake trout were also collected during other fall bottom trawling and biological data from these fish were included in other analyses but not in calculations of density.

Determining strain and age of lake trout

Lake trout indigenous to Lake Ontario were extinct by 1960 (Christie 1973) and hatchery-reared lake trout stocked since then have been distinguished by clipping fins and, rarely, maxillary bones. Most fish released in U.S. waters of Lake Ontario after 1980, and most fish released lakewide after 1986, had the adipose fin removed and a coded wire tag (CWT) injected in the snout (Elrod et al. 1995). Strains stocked over the study period included the Clearwater Lake, Manitoba, strain (CWL) from wild fish; the Seneca Lake, New York, strain (SEN) from wild fish and also from broodstock at the Allegheny National Fish Hatchery; the Lake Superior strain (SUP) from brood stock at the Marquette (Michigan) State Fish Hatchery; the Jenny and Lewis Lake, Wyoming, strain (JEN) from broodstock at the Jackson (Wyoming) National Fish Hatchery (1984–1987 year-classes) and at the Saratoga (Wyoming) National Fish Hatchery (1991–1992 year-classes); and the Lake Ontario strain (ONT) from hatchery-origin parents of mixed strains that survived to maturity in Lake Ontario. Age and strain could not be determined for most fish released in Canadian waters; therefore, we combined all Canadian-stocked fish that we recovered into a single multiage, multistrain group (CAN). Fish of unknown origin that had regenerated fins, had shed their CWT, or both were designated UNK.

Classifying sea lamprey marks

For live lake trout caught in gillnets and dead lake trout caught in trawls, we recorded total length (mm), clips, sex, and sea lamprey marks (except that total lengths of dead lake trout were not recorded in 1982). Live lake trout were also weighed; length–weight relations were calculated and used to estimate the weights of the dead lake trout at the time of death. Sea lamprey marks on live lake trout were classified according to the criteria of King (1980) as type A (skin at the attachment site broken and underlying musculature exposed) and healing stages I, II, III, and IV(hereafter referred to as stages A-1, A-2, A-3, and A-4) or as type B (skin at the attachment site not broken). An A-1 mark is a mark from which a sea lamprey has recently detached, either voluntarily or because the attack was interrupted by sampling. The A-1 mark heals to an A-2 mark, and those type-A marks with higher numerical designations are progressively older and in later stages of healing.

Only dead lake trout with A-1 or A-2 marks were counted as having succumbed to a sea lamprey attack. We combined the two mark categories because the transparent membranelike material distinguishing stage A-1 from A-2 (King 1980) was sometime difficult to see on dead fish due to post-mortem decomposition of tissue and consequently A-1 marks could not be separated from marks in the earliest stages of healing. However, further healing to stage A-3, as indicated by repigmentation at the margins of the mark (King 1980), was obvious and was interpreted as evidence of survival from a previous attack. The presence of stage A-1 or A-2 marks as a criterion for cause of death is justified by their nearly universal occurrence on dead lake trout and infrequent occurrence on live lake trout (Bergstedt and Schneider 1988).

Statistical analyses

To relate our observations of sea lamprey marks to density of dead lake trout, we needed to correctly assign the marks to the proper parasitic cohort of sea lamprey. Only one parasitic cohort is present in September. The previous cohort would have left the lake and spawned 3–4 months earlier in May or June (Bergstedt and Swink 1995). To investigate if stage A-2 or A-3 marks observed in September were inflicted by the present or the previous parasitic cohort of sea lamprey, we correlated A-2 and A-3 marks in each year with the number of A-1 marks observed that same year and with the number of A-1 marks observed the previous year.

We used the density of dead SUP lake trout in the 30- to 100-m stratum (the only stratum sampled every year) in correlations with the number of sea lamprey marks observed on live SUP fish from the gillnet surveys, using selected summaries of mark stages. Summaries of mark stages we used were: A-1 only; the sum of A-1 and A-2; and the sum of A-1, A-2, and A-3. These groups include progressively older attacks, ending with the summary marking statistic recommended by Eshenroder and Koonce (1984) for tracking annual changes in numbers of lake trout killed. The summary of sea lamprey marks that was most highly correlated was then used as the independent variable in a regression predicting density of SUP fish killed by sea lamprey, and in a multiple regression also considering host density as an independent variable. Host density was measured by the number of SUP lake trout ≥600 mm (sizes desirable to sea lamprey) caught in annual gillnet surveys.

Because susceptibility to sea lamprey attack and the lethality of an attack may be influenced by bathythermal distribution, we used ANOVA to compare the mean depths and water temperatures of capture of the various strains of lake trout. Mean depths and water temperatures of capture were calculated for lake trout ≥600 mm (desirable hosts) of each strain from gillnet catches in those years when 29 or more fish were caught. Sample sizes were large enough in 1982–1989 (CWL), 1988–1992 (SEN), and 1982–1992 (SUP). Comparisons between strains were made only for years where those periods overlapped. Catches from areas in the northeast portion of U.S. waters were not included. These areas are relatively shallow (mostly <35 m deep), and in those years when the epilimnion was thick, lake trout had no opportunity to descend to greater depths as they could in other areas of the lake.

The relation of length, strain, and year collected to the probability of encountering a live lake trout bearing an A-1 mark was examined with multiple logistic regression (Afifi and Clark 1990). The data set we analyzed included records from all live CWL, SEN, or SUP fish ($N = 13\,209$) captured during September gillnet surveys; each record (fish) included strain, length, year of collection, and occurence of an A-1 mark (yes = 1; no = 0). Multiple logistic regression was appropriate because it allowed the description of binomial data (presence or absence of marks) by both categorical data (genetic strain) and

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[3] Use of trade names does not constitute New York State or U.S. Government endorsement of commercial products.

Table 1. Number of dead lake trout caught in bottom trawls, density, and the estimated population in each of three depth strata in U.S. waters of Lake Ontario, October–November, 1982–1992 for dead lake trout bearing an A-1 or A-2 sea lamprey mark of all strains and (in parentheses) for dead lake trout of the SUP strain.

Year	Depth Stratum (m)	Catch	Area swept (ha)	Density (no./ha)	Population estimates	
					No.	95% CL
1982	30–100	(5) 12	39.1	(0.128) 0.307	(26 100) 62 600	32 300 – 108 400
	100–160	(1) 1	6.6	(0.152) 0.152	(36 600) 36 600	3 700 – 205 500
	>160		0		18 700*	
1983	30–100	(5) 6	138.1	(0.036) 0.043	(7 300) 8 800	3 200 – 19 300
	100–160		0		3 000*	
	>160		0		2 600*	
1984	30–100	(11) 16	69.4	(0.159) 0.231	(32 400) 47 100	27 000 – 76 400
	100–160	(1) 2	8	(0.125) 0.250	(30 100) 60 300	6 100 –218 000
	>160		0		14 000*	
1985	30–100	(8) 21	278.2	(0.029) 0.075	(5 900) 15 300	9 500 – 23 500
	100–160	(2) 2	21.4	(0.093) 0.093	(22 400) 22 400	2 300 – 81 500
	>160	(0) 0	12.1	(0) 0	(0) 0	0 – 90 900
1986	30–100	(14) 24	165.3	(0.085) 0.145	(17 300) 29 600	19 000 – 43 900
	100–160	(0) 0	47.6	(0) 0	(0) 0	0 – 18 800
	>160	(1) 1	40.6	(0.025) 0.025	(7 400) 7 400	700 – 41 200
1987	30–100	(6) 10	157.0	(0.038) 0.064	(7 800) 13 100	6 100 – 23 900
	100–160	(3) 3	38.2	(0.079) 0.079	(19 000) 19 000	3 800 – 56 000
	>160	(0) 0	40.0	(0) 0	(0) 0	0 – 27 500
1988	30–100	(6) 8	165.2	(0.036) 0.048	(7 300) 9 800	4 200 – 19 500
	100–160	(0) 0	41.7	(0) 0	(0) 0	0 – 21 500
	>160	(1) 1	41.1	(0.024) 0.024	(7 200) 7 200	700 – 40 500
1989	30–100	(3) 8	222.3	(0.013) 0.036	(2 700) 7 300	3 100 – 14 500
	100–160	(1) 1	48.6	(0.021) 0.021	(5 100) 5 100	500 – 27 900
	>160	(0) 1	22.0	(0) 0.045	(0) 13 400	1 400 – 75 700
1990	30–100	(8) 13	184.8	(0.043) 0.070	(8 800) 14 300	7 600 – 24 600
	100–160	(1) 1	44.7	(0.022) 0.022	(5 300) 5 300	500 – 30 300
	>160	(1) 1	42.5	(0.024) 0.024	(7 100) 7 100	700 – 39 200
1991	30–100	(17) 19	184.5	(0.092) 0.103	(18 800) 21 000	12 800 – 32 700
	100–160	(1) 1	50.8	(0.020) 0.020	(4 800) 4 800	500 – 27 000
	>160	(0) 0	45.0	(0) 0	(0) 0	0 – 24 400
1992	30–100	(2) 6	183.4	(0.011) 0.033	(2 200) 6 700	2 400 – 14 600
	100–160	(2) 3	48.7	(0.041) 0.062	(9 900) 14 900	3 000 – 43 800
	>160	(0) 0	39.1	(0) 0	(0) 0	0 – 28 100

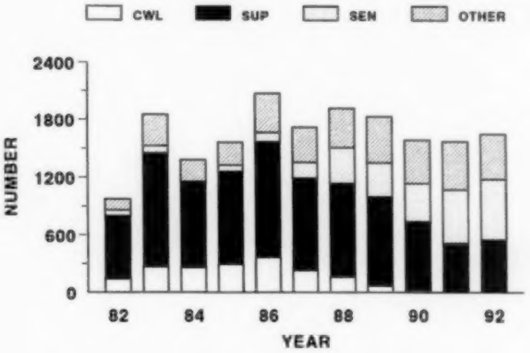
Note: Also shown is the amount of bottom area swept by the trawls in each depth stratum each year. Areas used to calculate population estimates from densities were 204 000 ha for the 30 to 100 m depth stratum, 241 000 ha for the 100 to 160-m stratum, and 297 000 ha for the >160 m stratum. Asterisks mark population estimates that were calculated from the density in a shallower stratum by use of the mean ratio of densities in the two strata in years when both strata were sampled. Confidence intervals (CI) are fiducial.

continuous data (year and fish length). The significance of the contribution of the independent variables and their interactions, to the logistic regression model was evaluated with G^2.

We tested for differences among strains in the lethality of sea lamprey attacks. Strains were paired and the ratios of live fish with A-1 marks to lake trout killed by sea lamprey were compared by use of Chi-square, 2 × 2 contingency tables.

Kolmogorov–Smirnov (two-sample) tests (Conover 1980) were used to compare length distributions of live lake trout with length distributions of lake trout killed by sea lamprey to determine if lamprey selected their prey by size. To determine if attack lethality varied with size of fish, we used Kolmogorov–Smirnov tests to compare length distributions of live lake trout bearing an A-1 mark with lake trout killed by sea lamprey. Because strain might affect size selection and lethality of attack and because only one strain was prevalent in our samples in all years, fish of that strain were the only ones used

Fig. 2. The catch of various strains of lake trout in gillnets in U.S. waters of Lake Ontario during September 1982–1992. CWL, Clearwater Lake; SUP, Lake Superior; SEN, Seneca Lake; other, all others.



in the Kolmogorov–Smirnov tests. Because of the small catch of live lake trout marked by sea lamprey and lake trout killed by sea lamprey each year, we pooled observations for adjacent years whenever Kolmogorov–Smirnov (two-sample) tests indicated that the lengths of live, sea lamprey marked lake trout available as prey were similar ($P > 0.05$). Length distributions were compared only when the smaller of the two sample sizes was ≥10.

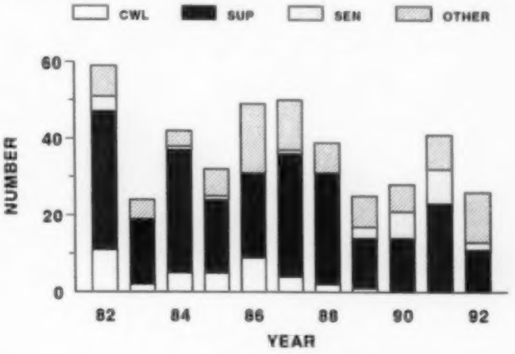
Results

We collected over 18 000 live (Fig. 2) and 256 dead lake trout (Table 2) during 1982–1992. The catch of live lake trout increased sharply in 1983, but thereafter, it fluctuated within a narrow range. The September–December catch of dead lake trout from all sources of mortality, including fish that were largely decomposed but still recognizable as lake trout, ranged from 42 in 1985 to 8 in 1983.

Live lake trout captured in gillnets were mostly of the CWL, SEN, and SUP strains and dead lake trout recovered in trawls were mostly of the CWL and SUP strains. The JEN strain was also captured in small numbers along with three mixed strains: ONT, CAN, and UNK. The SUP strain was prominent in the gillnet catch in all years, but their dominance diminished from 67% in 1982 to 23% in 1992 (Fig. 2). The CWL strain was conspicuous in the early and middle 1980s, and the SEN strain in the late 1980s and early 1990s (Fig. 2).

The water depths and temperatures where we captured live lake trout in gillnets differed among strains. The CWL strain fish were caught in shallower, warmer water than the SUP strain fish each year. For the 8-year period 1982–1989, the mean depth of capture of CWL strain fish (31.2 m) was significantly shallower ($P < 0.01$) than that of SUP strain fish (34.7 m) and the mean water temperature of capture (8.9°C) was significantly higher ($P < 0.01$) than that of SUP strain fish (7.8°C). In contrast, the SEN strain lake trout were caught in deeper, colder water than the SUP strain lake trout each year. For the 5-year period 1988–1992, the mean depth of capture of SEN strain fish (43.2 m) was significantly deeper ($P < 0.01$) than that of SUP strain fish (37.2 m) and the mean temperature of capture (6.4°) was significantly lower ($P < 0.01$) than that of SUP strain fish (7.4°C).

Fig. 3. Numbers of type A-1 sea lamprey marks on various strains of lake trout caught in gillnets in U.S. waters of Lake Ontario in September 1982–1992. CWL, Clearwater Lake; SUP, Lake Superior; SEN, Seneca Lake; other, all others.



Sea lamprey marks and density of dead lake trout

All classifications of sea lamprey marks were present on live SUP lake trout each year, during 1982–1992. The mean number and range of sea lamprey marks in each class and in annual observations were

	Type A stage				
	I	II	III	IV	Type B
Mean	23	24	113	292	57
Range	11–37	10–56	31–189	95–466	2–213

Among the type A marks on SUP fish, A-1s peaked in 1982, A-2s in 1985, A-3s in 1988, and A-4s in 1986. Type B marks peaked in 1986 at nearly 2.5 times the next highest value, which was recorded in 1989. The number of A-1 marks on live lake trout (mostly SUP lake trout) declined between 1982 and 1992 (Fig. 3).

The majority of A-3 marks observed in September were likely caused by the previous parasitic cohort of sea lamprey. Numbers of A-3 marks were significantly correlated with the number of A-1 marks the previous year ($r = 0.65$, $N = 10$, $P = 0.04$), but not with A-1 marks in the current year ($N = 11$, $P > 0.05$). Numbers of A-2 marks were not correlated with A-1 marks in either the previous or the current year ($N = 10$, $P > 0.05$; $N = 11$, $P > 0.05$).

The density of sea lamprey killed lake trout on the lake bottom was related to the number of sea lamprey marks observed, but only if marks in the earliest stage of healing (A-1) were considered. Density of SUP fish killed by sea lamprey October through mid-November was best correlated with the number of A-1 marks on live SUP fish in the September gillnet surveys ($r = 0.69$, $N = 11$). If the sum of A-1 and A-2 marks were used, the correlation was weaker ($r = 0.35$, $N = 11$). If the sum of A-1, A-2, and A-3 marks was used, the correlation was negative ($r = -0.40$, $N = 11$). A linear regression of the density of sea lamprey killed lake trout on the number of A-1 marks in September was significant

Table 2. Numbers and lengths of dead lake trout of various strains recovered in U.S. waters of Lake Ontario during September–December 1982–1992.

		Marked			Not marked		Decomposed		
Year	Strain	*n*	Mean	Range	*n*	Length	*n*	Mean	Range
1982	SUP	6			0		0		
	UNK	7			0		0		
1983	SUP	6	598	488–680	0		0		
	UNK	1		654	0		1		560
1984	SUP	18	598	430–703	0		0		
	CAN	3	605	562–687	0		0		
	UNK	4	648	558–724	0		0		
1985	SUP	11	647	498–725	0		3	667	615–740
	CWL	4	615	555–667	0		0		
	CAN	7	661	597–718	0		0		
	UNK	9	655	585–722	0		8	648	540–760
1986	SUP	18	661	492–780	0		1		603
	CWL	3	689	676–705	0		1		680
	CAN	6	683	653–740	0		0		
	UNK	6	687	623–745	1	650	3	655	575–710
1987	SUP	15	668	562–783	1	675	2	725	700–750
	CWL	2	695	689–701	0		0		
	CAN	3	739	665–788	1	612	1		692
	UNK	2	741	725–757	1	718	2	693	685–700
1988	SUP	9	707	666–775	0		2	685	680–690
	CWL	0			1	725	0		
	CAN	1		656	0		0		
	UNK	3	689	661–716	0		4	686	645–750
1989	SUP	4	690	634–765	1	681	2	725	700–750
	CAN	3	666	546–768	0		0		
	UNK	3	773	740–810	1	660	1		660
1990	SUP	10	715	580–755	0		0		
	SEN	0			1	267	0		
	CAN	1		586	0		0		
	JEN	1		552	0		0		
	UNK	3	685	568–809	0		2	739	727–750
1991	SUP	18	716	605–823	0		4	676	590–717
	SEN	0			1	655	1		695
	CAN	1		738	0		0		
	UNK	1		800	0		3	670	590–770
1992	SUP	4	774	663–843	0		3	680	645–705
	CWL	1		715	0		0		
	SEN	1		590	0		0		
	CAN	1		733	1	761	0		
	JEN	1		665	0		2	662	650–673
	UNK	2	597	570–623	0		1		690

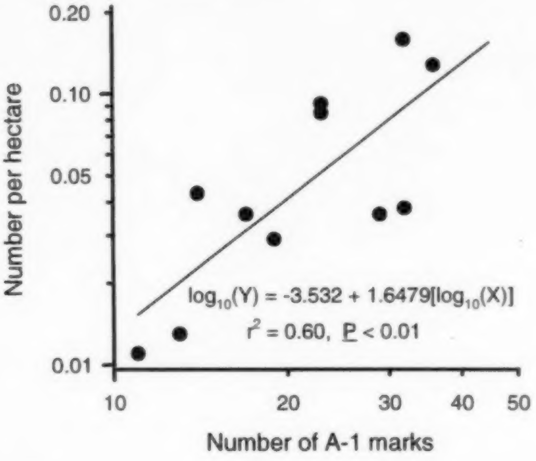
Note: Dead lake trout were assigned to one of three classes: marked, with a type A stage I or II sea lamprey mark (presumably killed by a sea lamprey); not marked, no type A marks; and decomposed, presence or absence of a sea lamprey mark could not be determined (may or may not have been killed by sea lamprey). CWL, Clearwater Lake; SUP, Lake Superior; JEN, Jenny Lake; SEN, Seneca Lake; CAN, all Canadian stocked fish; UNK, all others.

($r^2 = 0.48$, $N = 11$, $P < 0.02$), but inspection of the residuals suggested that the relationship was not linear. Indeed, a $\log_{10}$ transformation of both variables resulted in a higher r^2 (Fig. 4; $r^2 = 0.60$, $P < 0.01$). Addition of a second independent variable (abundance of SUP lake trout ≥600 mm) did not result in a significant increase in r^2 ($P = 0.19$), although the coefficient was negative suggesting that attacks might result in fewer deaths as the abundance of host fish rose.

Dead lake trout

Of the 256 dead lake trout (all strains) we recovered during October–December 1982–1992, one hundred and ninety-nine

Fig. 4. For Lake Superior strain lake trout in U.S. waters of Lake Ontario, the regression of the logarithm of the number per hectare of lake trout killed by sea lamprey in October–November at depths of 30–100 m on the logarithm of the number of type A-1 marks on live fish in September gillnet catches.

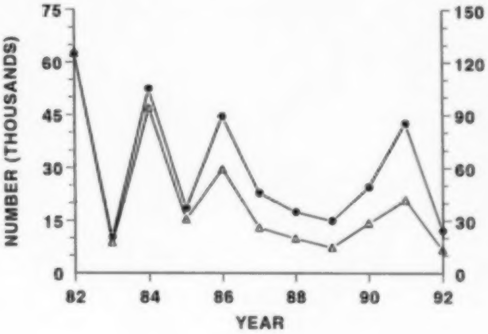


were killed by sea lamprey, 47 were too decomposed to determine the cause of death, and 10 died from other causes (Table 2). During 1982–1985, all 76 intact dead fish recovered bore A-1 or A-2 marks. After 1985, sea lamprey still accounted for most dead fish recovered, as 123 of the 133 intact fish (92%) bore an A-1 or A-2 mark. Of the 10 lake trout that died from causes other than a sea lamprey attack, all were collected after 1985 and one had an obvious hooking injury. Lake trout killed by sea lamprey were 60% SUP strain, 5% CWL, <1% SEN, and 34% other (mostly CAN and UNK). Lake trout that died from causes other than a sea lamprey attack were 20% SUP, 20% SEN, 10% CWL, and 50% other (mostly CAN and UNK). Lake trout too decomposed to determine cause of death were 40% SUP, 2% SEN, 2% CWL, and 56% other (mostly UNK).

The estimated number of lake trout killed by sea lamprey during October 1 – November 15 ranged from 17 100 in 1988 to 121 400 in 1984 and, in general, sea lamprey killed more lake trout prior to 1985 (Table 1). These numbers are underestimates because they do not account for fish that may have been killed by sea lamprey prior to our survey (some of the lake trout recovered during October 1 – November 15 were too decomposed to determine cause of death) or for lake trout killed after November 15. These estimates for 1982–1985 are lower than previously reported (Bergstedt and Schneider 1988), because the area swept by the trawls was subsequently found to be larger.

Mean length of the lake trout killed by sea lamprey increased as the study progressed (Table 2). Because size of the trout that sea lamprey killed rose as the numbers killed declined, the biomass of lake trout lost annually to sea lamprey did not fall as rapidly as numbers. For example, in the 30- to 100-m depth stratum the mean annual number of fish killed during 1990–1992 was 65% less than during 1982–1984, whereas the mean annual biomass of fish killed during 1990–1992 was only 37% less than during 1982–1984 (Fig. 5).

Fig. 5. Estimated numbers (triangles) and biomass (circles) of lake trout killed by sea lamprey (all strains combined) in the 30- to 100-m depth stratum in U.S. waters of Lake Ontario, October 1 – November 15, 1982–1992.



Lake trout killed by sea lamprey were most frequently recovered in the 30- to 100-m depth stratum, followed by the 100- to 160-m stratum, and the ≥160-m stratum (Table 1). The ≥160-m stratum encompassed the most bottom area and the 30- to 100-m stratum encompassed the least amount of bottom area.

Size of hosts selected by sea lamprey

We rarely found A-1 marks on live lake trout <400 mm long, but they were increasingly common on longer fish:

Size group (mm)	Fish examined	Number with A-1 marks	Percent marked
<400	2 145	1	0.05
400–499	2 505	16	0.6
500–599	3 185	49	1.5
≥600	10 307	349	3.4

Fully 84% of all A-1 marks observed were on lake trout ≥600 mm long, even though fish ≥600 mm contributed only a small portion of the gillnet catch prior to 1986 (Fig. 6).

The size of lake trout available to sea lamprey increased during the study period, as indicated by the median lengths of live SUP lake trout caught in our gillnet surveys (Fig. 7A). Comparisons of length distributions of live SUP fish during 1983–1992 indicated significant differences (Kolmogorov-Smirnov, two-sample tests, $P < 0.01$) between all adjacent years except 1991 and 1992 ($P > 0.05$). Because the sizes of prey available to sea lamprey were similar during 1991–1992, we combined the data for those years.

Lengths and ages of SUP fish killed by sea lamprey and recovered with bottom trawls rose concurrently with the lengths of live SUP lake trout caught in gillnets during 1983–1992 (Figs. 7A and Fig. 8). The median length of dead fish was greater each year except 1989, a year when only four dead fish were recovered. The length distributions of the dead lake trout were significantly different (Kolmogorov–Smirnov, two-sample tests, $P < 0.05$) from the those of the

Fig. 6. Number of lake trout ≥600 mm (by strain) caught in gillnets in U.S. waters of Lake Ontario during September 1982–1992. CWL, Clearwater Lake; SUP, Lake Superior; SEN, Seneca Lake; other, all others.

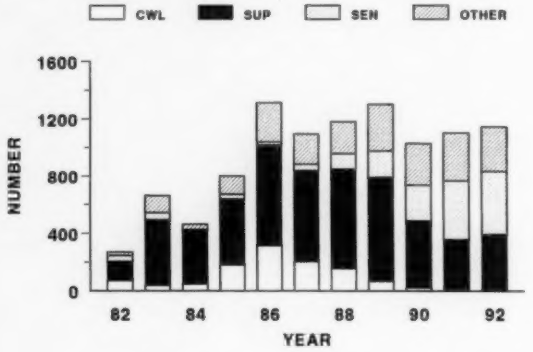


Fig. 7. The median lengths (horizontal line), range between the 25th and 75th percentiles (rectangle), and range of lengths (vertical line) for (A) live lake trout (stippled rectangles) and lake trout killed by sea lamprey (open rectangles) and (B) live lake trout that had type A-1 sea lamprey marks (stippled rectangles) and lake trout killed by sea lamprey (open rectangles). All lake trout were Lake Superior strain. Live fish were caught with gillnets in September; lake trout killed by sea lamprey were collected with bottom trawls during September–December, Lake Ontario, 1983–1992 (data combined for 1991 and 1992; see text for details). Above each year we listed the sample size for live lake trout, sample size for dead lake trout and the results of a Kolmogorov–Smirnov, two-sample test performed when both sample sizes were ≥10. ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$.

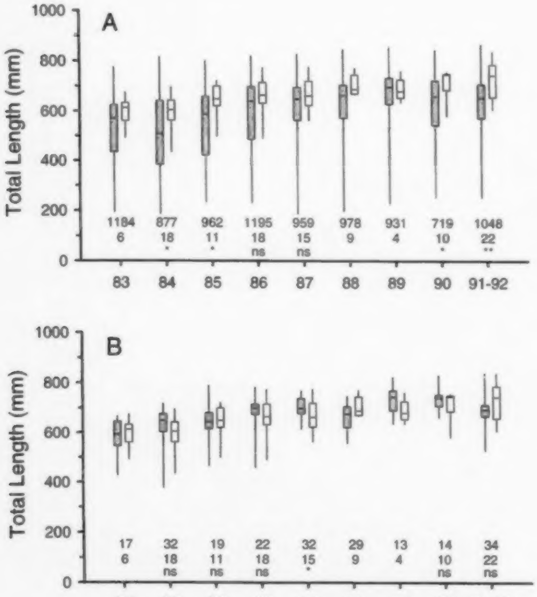
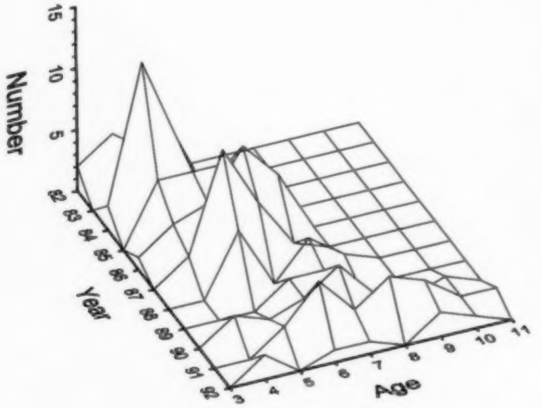


Fig. 8. The number of lake trout killed by sea lamprey (all strains combined) collected with bottom trawls in U.S. waters of Lake Ontario in September–December 1982–1992 by age and year of collection.



live lake trout in four of six comparisons (Fig. 7A). Sea lamprey killed mostly age-3 and -4 lake trout during 1982–1984, mostly age-5 and -6 trout during 1985–1988, and mostly age-6 and older trout during 1989–1992 (Fig. 8).

Probability of a sea lamprey attack

The year of collection, length, and strain all contributed significantly ($P < 0.05$) to a multiple logistic regression model predicting the probability of attack by a sea lamprey. None of the interactions among these variables was significant. The best model was

$$\text{logit}(p_{\text{A-1}}) = -2.923 - 0.375\text{CWL} - 0.899\text{SEN} - 0.064Y + 0.00748L$$

where $p_{\text{A-1}}$ is the probability of bearing an A-1 mark, CWL and SEN are dummy variables for the CWL and SEN strains (comparisons are to the SUP strain used as the standard), Y is year of collection, and L is total length in millimetres.

The parameters in the model are best interpreted as log odds ratios. Detransforming from the logit scale, a CWL lake trout was 0.69 times as likely to bear an A-1 mark as SUP fish. The 95% confidence interval was 0.45–1.04, indicating that marking of those strains was not significantly different. A SEN lake trout was 0.41 times as likely to bear an A-1 mark as a SUP. The 95% confidence interval in this case was 0.26–0.64, indicating that the probability of marking for SEN fish was significantly lower than for SUP fish. The parameters for year and length were interpreted based on an incremental change of one unit. The proportion of sea lamprey marked fish increased by a factor of 1.008 for each millimeter of length and decreased by a factor of 0.94 relative to the previous year. In this analysis the parameter for year was included to account for the decrease in marking due, we presume, to decreasing numbers of sea lamprey during 1982–1992 and is useful only in describing probability of marking in this data set. The odds ratios for strain and length, however, describe basic relations that may be similar in other multistrain populations.

Table 3. Chi-square contingency tables comparing the ratios of live fish bearing a type A-1 sea lamprey mark to lake trout killed by sea lamprey among three strains of lake trout in Lake Ontario during 1982–1992.

Strain	Live	Dead	Total and live/dead ratio	Strain	Live	Dead	Total and live/dead ratio	Strain	Live	Dead	Total and live/dead ratio
SEN	28	1	29	SEN	28	1	29	SUP	247	119	366
	(20)	(9)	28.00		(25)	(4)	28.00		(252)	(114)	2.08
SUP	247	119	366	CWL	39	10	49	CWL	39	10	49
	(255)	(111)	2.08		(42)	(7)	3.90		(34)	(15)	3.9
Total	275	120		Total	67	11		Total	286	129	
$\chi^2 = 10$, $P = 0.00$				$\chi^2 = 4.3$, $P = 0.03$				$\chi^2 = 2.9$, $P = 0.08$			

Note: Numbers in parentheses are expected values.

Lethality of a sea lamprey attack

Lengths of SUP lake trout killed by sea lamprey were similar to the lengths of live SUP lake trout with A-1 marks caught in gillnets, suggesting that lamprey attacks were equally lethal across the size range of lake trout attacked (Fig. 7B). The length distributions of lake trout killed by sea lamprey and live, sea lamprey marked fish were not significantly different (Kolmogorov–Smirnov, two-sample tests, $P > 0.05$) in five of six comparisons. Furthermore, the ranges in lengths were similar between live, sea lamprey marked fish and dead fish in all 9 years, and neither group consistently had the greatest median length.

A sea lamprey attack was less likely to kill SEN lake trout than CWL or SUP lake trout. Ratios of live, A-1 marked fish to fish killed by sea lamprey were 2.1:1 for SUP fish, 3.9:1 for CWL fish, and 28:1 for the SEN fish (Table 3). Ratios for the SUP and CWL strains were not significantly different ($P = 0.09$), but the ratio for the SEN strain differed ($P < 0.05$) from those for either the SUP and CWL strains.

Male SUP lake trout died from sea lamprey attacks at the same rate as female SUP lake trout. Among the live SUP lake trout with A-1 marks there were 109 males and 99 females, and among SUP fish killed by sea lamprey there were 25 males and 21 females. The sex ratio of live SUP fish with an A-1 mark was not different from the sex ratio of SUP fish killed by sea lamprey ($\chi^2 = 0.06$, $P = 0.81$).

Discussion

Sea lamprey marks as measures of lake trout loss

Abundant evidence shows that sea lamprey devastated the lake trout in the Great Lakes (e.g., Coble et al. 1990). However, the evidence largely consists of relating observed rates of sea lamprey marks to either trends in lake trout abundance (e.g., Fry 1953; Eschmeyer 1957) or to various population statistics (e.g., Budd et al. 1969; Pycha 1980). In a different approach, Spangler et al. (1980), estimated attack lethality from the accumulation of healed sea lamprey marks on recaptured members of a group of tagged lake whitefish (*Coregonus clupeaformis*). The methods used by previous authors were necessary because fish killed by attacks were never seen (Spangler et al. 1980) and inferences had to be based on samples of the survivors. Here, we have shown that the number of observed sea lamprey marks can also be quantitatively related to the population density of fish that did not survive. Although this finding is not remarkable given the other evidence that sea lamprey kill lake trout (Coble et al. 1990), it provides confirmation based on samples of the fish that succumbed to attacks and makes very different assumptions.

In data collected in autumn, A-1 marks are preferrable for indexing the current year's loss of lake trout to sea lamprey activity. Eshenroder and Koonce (1984) recommended autumn collections and suggested that the best indicator of the destructive activity of a single cohort of parasitic sea lamprey was the total number of A-1, A-2, and A-3 marks combined. The justification for combining the three stages was that increased sample size would increase the power of the analysis. Though not specifically stated, they probably assumed that marks caused by the previous parasitic cohort would have healed to stage A-4 and would not be included in the sample. Combining mark stages A-1, A-2, and A-3 is not indicated by our findings that only A-1 marks were related to the density of dead lake trout and by the lack of correlation between either A-2 or A-3 marks and A-1 marks in the same year. The strong correlation between A-3 and A-1 marks in the previous year suggests that many A-3 marks observed in September were caused by the outgoing parasitic cohort that spawned in spring of that year. Furthermore, King (1980) presented data (in the figure captions) that indicate that marks may take several months to heal to the A-3 stage. The statistical power gained by using marks older than A-1 may not be useful if their inclusion confounds analyses by including the activity of more than one parasitic cohort of sea lamprey.

Our success at relating sea lamprey marking to losses of lake trout was probably due to the use of sea lamprey marking data collected in autumn, as suggested by Eshenroder and Koonce (1984), and to King's (1980) classification system, which allowed us to count only the most recent sea lamprey marks. Collections of lake trout killed by sea lamprey (Bergestedt and Schneider 1988) confirmed Kitchell and Breck's

(1980) hypothesis that most sea lamprey induced mortality occurs in the fall. Analysis of the growth of parasitic-phase sea lamprey also shows that growth increases sharply in mid-September and is greatest from early October to mid-November (Bergstedt and Swink 1995). Because marking rates vary widely through the year (Jacobson 1989), it is important to use a measure of sea lamprey activity that reflects the number of attacks made during autumn. In our September gillnet collections, use of only A-1 marks ensured that the attacks were either recently completed or interrupted, ongoing attacks that would reflect as closely as possible sea lamprey activity during the period of highest mortality.

Size-selective predation

Field observations of sea lamprey marks have long shown that attack rate increases as prey size increases for several host species such as white suckers (*Catostomus commersoni*; Hall and Elliott 1954), lake trout (Eschmeyer 1957), rainbow trout (*Oncorhynchus mykiss*; Berst and Wainio 1967), and Atlantic salmon (*Salmo salar*; Beamish 1980). Two concepts emerged to explain why smaller fish were more likely to be killed by an attack or that sea lamprey selectively attacked larger fish. Initially, Eschmeyer (1957) concluded that the lower attack rate for small lake trout reflected a higher mortality per attack for small fish rather than a preference by sea lamprey for large fish. Swink (1990) demonstrated that attacks on small (469–557 mm) lake trout were more lethal than attacks on larger specimens, providing some support for this explanation. However, Budd and Fry (1960) and Pycha and King (1975) concluded from population studies that the lower sea lamprey marking rates on small lake trout occurred because sea lamprey preferred larger fish. Preference of sea lamprey for larger prey has also been shown in several laboratory studies (Farmer and Beamish 1973; Cochran 1985; Swink 1991).

We confirmed size-selective attack by sea lamprey by analyses of field data that compare the size distributions of available lake trout to (1) those attacked by sea lamprey and (2) those killed by the attack. Our findings indicate that the probability of attack increased significantly as lake trout size increased; the large majority of A-1 marks were on live fish ≥600 mm whereas live lake trout <400 mm were typically never marked when larger lake trout were available. The low number of sea lamprey marks on small lake trout does not result from higher mortality of small fish, because few small, dead fish were captured in trawls. Furthermore, the similarity of the lengths of live, A-1 marked fish and lake trout killed by sea lamprey (Fig. 7B) strongly suggests that our trawls recovered all sizes of dead lake trout equally well. Swink (1991) observed that sea lamprey in the laboratory generally avoid lake trout <600 mm when larger specimens were available, agreeing well with our observation that 84% of attacks were on fish ≥600 mm.

Our study eliminated two factors that normally prevent analysis of host size selection by sea lamprey in the field. Cochran (1985) noted that the potential for higher mortality of smaller lake trout and the longer exposure to attack for larger, older lake trout are seldom considered in field studies. We were able to rule out higher mortality of small lake trout by sampling fish that did not survive. The problem of older fish being exposed for more years to sea lamprey was avoided by considering only A-1 marks on live, sea lamprey marked fish and A-1 or A-2 marks on dead fish in our fall collections. This ensures the inclusion of attacks made only by the current cohort of parasitic-phase sea lamprey (King 1980).

The similarity of lengths of live, A-1 marked fish and dead lake trout implies (Fig. 7B) that there was no survival advantage for large lake trout, at least with the size structure in Lake Ontario through 1992. Laboratory studies (Swink 1990) showed lower survival of small(469–557 mm) lake trout from a single sea lamprey attack, but no difference in survival between medium (559–650 mm), and large (660–825 mm) lake trout, confirming our observations. Farmer et al. (1975) showed that the probability of death is related to the proportion of a host's blood that is removed and suggested that a large enough host could sustain an attack indefinitely. We do not dispute that conclusion, but note that size confered no survival advantage over the size range of lake trout in this study.

Lake trout mortality

Our estimates of lake trout lost to predation by sea lamprey in U.S. waters of Lake Ontario should be considered minimal for two reasons. First, we do not know what proportion of the decomposed lake trout recovered in trawls during October through mid-November were killed by sea lamprey. However, we suspect the proportion is high because most of the dead lake trout that were not decomposed were killed by sea lamprey. Second, we do not know how many lake trout were killed by sea lamprey after mid-November. Some dead lake trout with an A-1 mark have been recovered after this date during limited work. Inclement weather after mid-November precludes the regular scheduling of ship time. Finally, readers are cautioned that estimates of density of sea lamprey killed lake trout apply only to southern Lake Ontario, and densities in northern Lake Ontario may well be lower. Elrod et al. (1995) reported that catches of lake trout in gillnets were lower in northern than southern Lake Ontario. Rocky substrate along most of the north shore precludes bottom trawling.

Kitchell and Breck (1980) hypothesized that the length of time sea lamprey are attached to a host is negatively correlated with host abundance. If true, sea lamprey would feed longer on each host when lake trout are scarce, thereby removing greater blood volume and increasing the probability of killing the host. Conversely, when lake trout are abundant, sea lamprey would switch hosts more frequently, thus lessening blood loss and subsequent mortality for each host. Although we found that the sign of the coefficient for lake trout (host) abundance in a multiple regression predicting the density of dead lake trout was negative, agreeing with Kitchell and Breck's (1980) hypothesis, the additional variation explained in our data set by adding host abundance as an independent variable was not significant. Perhaps the contrast in lake trout density was insufficient to elicit a strong behavioral response from sea lamprey. Abundance of SUP lake trout ≥600 mm varied about fivefold during the study period.

Strain selection

A SEN strain lake trout was less likely to be attacked by sea lamprey than a SUP strain lake trout in southern Lake Ontario during 1982–1992. Two aspects of SEN behavior may have contributed to the different attack rates: bathythermal distribution and avoidance. The SEN lake trout occupied deeper, colder water than SUP lake trout. Greater overlap in bathythermal distributions of SUP lake trout and sea lamprey in fall might account for the higher attack rate among SUP fish. Alternatively, because sea lamprey were thought to be endemic to the Finger Lakes and Lake Ontario, SEN lake trout may have become relatively resistant to sea lamprey attacks (Christie 1973). A behavioral avoidance response to approaching sea lamprey could be among the mechanisms evolved during thousands of years of association. However, many believe sea lamprey were not endemic, but that they entered the Finger Lakes or Lake Ontario from the Hudson River via New York's Erie Canal in the mid-1800s (Aron and Smith 1971; Pearce et al. 1980; Smith 1995). Thus, the probability of producing a sea lamprey resistant strain of lake trout through natural selection is less conceivable, and other factors, including divergent depth distributions, are more likely responsible for the lower attack rates on SEN fish.

Not only are SEN fish less likely to be attacked than SUP fish, but we also found that they are more likely to survive a sea lamprey attack than SUP lake trout. In laboratory studies, however, Swink and Hanson (1986) found no difference between the two strains in their ability to survive a single sea lamprey attack, but speculated that the difference in survival in Lake Ontario was based on a difference in bathythermal distribution. Swink and Hanson (1989) and Swink (1993) found that lake trout survived sea lamprey attacks better at lower temperatures. Therefore, the fact that SEN fish occupy colder water than SUP fish in fall in Lake Ontario may, in part, explain the better survival of SEN strain lake trout attacked by sea lamprey.

Regardless of the advantage SEN fish have in coexisting with sea lamprey in Lake Ontario, multistrain stocking policies offer several advantages to the future management of sea lamprey and lake trout in the Great Lakes. Aside from lower attack rate and lethality of attack for SEN lake trout, another reason why SEN fish may have been less frequently attacked by sea lamprey was the availability of other suitable hosts; SUP fish were abundant and provided a buffer to feed residual populations of sea lamprey. If only SEN fish were stocked without a buffer, sea lamprey might increase their attacks on SEN, resulting in higher mortality. Moreover, if no remnant populations of native lake trout remain, other strains should continue to be stocked to promote genetic diversity (Schneider et al. 1983) until a self-sustaining wild population is established.

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Population genetics of Atlantic salmon (*Salmo salar*) in the River Shannon system in Ireland: an appraisal using single locus minisatellite (VNTR) probes

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Abstract: Single locus minisatellite DNA analysis was applied to a population genetic study of Atlantic salmon (*Salmo salar)* from the River Shannon system in Ireland. Following the construction of a hydroelectric scheme near the base of the 340 km long river in 1929, an extensive supplemental stocking programme commenced in 1960 (initially using Canadian ova). Samples of up to 100 wild parr were collected from nine different tributaries, with temporal sampling from three of these tributaries. A total of 1252 fish were screened for four minisatellite loci, using three minisatellite probes (p*Ssa*-A45/1, p*Ssa*-A45/2, and p*Str*-A9). Three of the loci were highly variable (*Ssa*-A45/1, *Ssa*-A45/2/1, and *Str*-A9), revealing 9, 10, and 8 resolvable alleles, respectively, when all samples were considered. Mean heterozygosities per locus were 0.61, 0.67, and 0.71 for the three loci, respectively. While the salmon from the tributaries below the hydro-electric scheme appeared distinct from those above, the differentiation detected in the upper system was attributed primarily to genetic drift resulting from poor escapement of adult salmon to some of the upper tributaries. No Canadian-specific alleles were detected at the fourth locus (*Ssa*-A45/2/2).

Résumé : Au moyen d'une analyse de l'ADN minisatellite à locus unique, nous avons étudié la génétique des populations de saumon atlantique (*Salmo salar)* du bassin du Shannon, en Irlande. Depuis la construction d'installations hydroélectriques près de l'embouchure de ce fleuve de 340 km (en 1929), un vaste programme d'empoissonnement complémentaire a été entrepris en 1960 (initialement au moyen d'ovules canadiens). Nous avons prélevé dans neuf affluents du fleuve des échantillons renfermant au moins 100 tacons sauvages, avec échantillonnage temporel dans le cas de trois affluents. Nous avons ainsi soumis 1252 poissons à un dépistage visant quatre locus minisatellites, au moyen des trois sondes à minisatellite p*Ssa*-A45/1, p*Ssa*-A45/2 et p*Str*-A9. Trois des locus étaient très variables (*Ssa*-A45/1, *Ssa*-A45/2/1 et *Str*-A9), révélant ainsi la présence de 9, 10 et 8 allèles séparables, respectivement, pour l'ensemble des échantillons. Les trois locus présentaient respectivement des taux moyens d'hétérozygotie de 0,61, 0,67 et 0,71. Les saumons des affluents situés en aval des installations hydroélectriques semblent distincts de ceux des affluents situés en amont, mais cette différence est principalement attribuable à la dérive génétique résultant du taux d'échappée médiocre qui caractérise les saumons adultes de certains affluents situés en amont. Aucun allèle spécifique au stock canadien n'a été détecté en ce qui concerne le quatrième locus (*Ssa*-a45/2/2).
[Traduit par la Rédaction]

Introduction

Protein electrophoresis studies have formed the basis of much of the literature describing genetic differentiation within and among species of fishes over the past two decades. Unfortunately, Atlantic salmon (*Salmo salar*), which is very valuable to both fisheries and aquaculture, has relatively few polymorphic loci detectable by allozyme electrophoresis (Allendorf and Utter 1979; Wilson et al 1995). Ståhl (1987) reported that just three allozyme loci accounted for more than 90% of the genetic diversity of the species based on samples collected from throughout the range. One of these (*MEP-2**) is

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probably affected by selection (Verspoor and Jordan 1989), potentially limiting its use as a population marker. While a number of other polymorphic loci occur, the low frequency of the variant alleles mean that they contribute little to the discriminatory power of the technique. This factor has severely curtailed attempts to differentiate between Atlantic salmon populations and to determine their relationships and interactions.

One of the most promising advances for detecting genetic variability in Atlantic salmon, has been the development of single-locus minisatellite DNA probes. As a result a number of highly variable, multi-allelic minisatellite loci can now be routinely studied (Taggart and Ferguson 1990; Bentzen et al. 1991; Prodöhl et al. 1994*a*; Taggart et al. 1994; Taylor et al. 1994). Minisatellite loci are regions of DNA consisting of multiple (two to several hundred) tandem repeats of a relatively short (9–100 base pairs) nucleotide sequence. Many potential allelic forms may exist at a locus, differing in the number of repeat units present (reviewed by Tautz 1993; Wright 1993). Analysis of the genetic composition at a number of minisatellite loci, termed DNA profiling (Jeffreys et al. 1991; Jeffreys and Pena 1993), detects greater levels of genetic differentiation than either allozyme or mitochondrial DNA methods (Flint et al. 1989).

Although the exact role of the core sequence common to many forms of minisatellite repeats is as yet unknown, the existence of proteins capable of binding to this core sequence (Epplen et al. 1993), together with evidence of associations between minisatellite loci and disease traits (Krontiris et al. 1993) suggest that these loci are not necessarily immune from selection. However, in the absence of any evidence for either direct functional roles or associations with loci undergoing selection, it is reasonable from an analytical perspective to regard minisatellite loci as selectively neutral, as defined by Kimura (1986).

When the Shannon hydroelectric scheme was originally constructed (1925–1929), it was assumed that salmon would ascend the system by way of the boat lock at Ardnacrusha power station or via the natural River Shannon, and a conventional pool and traverse fish pass was incorporated in the regulating weir at Parteen to facilitate this upstream migration (Fig. 1). However, it soon became apparent that fish were entering the tailrace in large numbers as far as the generating station and subsequently remaining in the tailrace. Salmon continued to use the natural channel route during the 1930s to 1950s, and evidence for this was collected by Went (1962) who intercepted, for tagging purposes, a total of 5461 smolts in the boat lock at Ardnacrusha in the years 1953–1955. The construction of a unique Borland-type fish lift at Ardnacrusha in 1959 provided upstream passage at the power station for the first time.

In the 1960s, restocking of the river commenced using stocks reared in a large enhancement hatchery. Although nonnative ova (including 120 000 ova from Canada in 1967) were used in the initial years, a native stock was soon established from brood stock collected from one of the tributaries (the Mulkear) entering the main channel below the dam. To date, however, there has been little monitoring of this considerable stocking effort (Table 1). For example, the relative return rates of the wild and hatchery fish have not been adequately quantified by tagging studies. As such, the only information on the present-day population structure of Atlantic salmon in the Shannon system comes from an allozyme study (Galvin et al. 1994).

The role of population genetic studies, regardless of the technique employed, is to describe genetic variability within and genetic differentiation among populations. In the present study, this information is required to provide guidelines on how the salmon in a river system should be best managed to conserve genetic integrity, especially where exploitation or enhancement is being undertaken. Difficulties associated with the detection of adequate levels of genetic variability and the interpretation of the adaptive significance of this variability has limited the usefulness of population genetics to fishery managers. While novel molecular techniques allow detection of high levels of genetic variability, the latter difficulty cannot easily be solved directly. However, an indirect approach of applying the null hypothesis that the variation at minisatellite DNA loci is largely moulded by stochastic factors alone is a valid alternative and is used here.

The aim of the present study was to analyse the genetic relationships within and among the salmon populations of the tributaries of the River Shannon and the strain from the enhancement hatchery, using single locus minisatellite DNA probes. A comparative analysis of the minisatellite data with the earlier allozyme results (Galvin et al. 1994) is beyond the scope of this investigation and will be reported as a separate analysis shortly.

Materials and methods

Sample collection

Samples of up to 100 wild juvenile Atlantic salmon were collected between 1991 and 1993 by electrofishing from the nine most important salmon-producing tributaries (Fig. 1). A comprehensive electrofishing survey carried out during 1990–1992 at 362 sites throughout the Shannon catchment area showed that, with the exception of the Rath River (a tributary of the Inny), salmon were absent from the upper 3000 km^2 of the Shannon catchment (M. O'Farrell, Department of Zoology, University College, Galway, and M. O'Grady, Central Fisheries Board, Dublin, unpublished data). Temporal stability of allele frequencies was assessed by sampling different yearclasses in the Cappagh, Blackwater, and Brosna tributaries. In the case of the Cappagh river, 0+ salmon collected in 1991 and again in 1993 were compared, while in the Blackwater and Brosna tributaries, samples consisting of 0+ and older parr, distinguishable by length, were collected in the autumn of 1992 and again in the spring of 1993. All tributary samples were collected from areas that had not been stocked in the previous 2 years to ensure that all fish were naturally spawned. However, it is likely that some of these naturally spawned fish were the progeny of adult returns from earlier stocking programmes. A sample of 200 1+ smolts was collected from the enhancement hatchery in April of 1992. Fish were either killed instantly and transported on dry ice to a –70°C freezer at the laboratory for later dissection, or transported live to the laboratory where tissue was taken from freshly killed specimens. Once dissected, tissues were stored at –20°C for up to 18 months prior to extraction of DNA.

DNA analysis

DNA was extracted from white skeletal muscle using a rapid phenol–chloroform extraction protocol described by Taggart et al. (1992). Four micrograms of DNA was digested overnight at 37°C with *Pal*I restriction enzyme (6U/μg DNA) in a total volume of 18 μL. Each of the samples was run with an internal marker (Taggart

Fig. 1. River Shannon system in Ireland depicting the locations of the main salmon tributaries, the sites sampled for the study, and a magnification of the hydroscheme section.

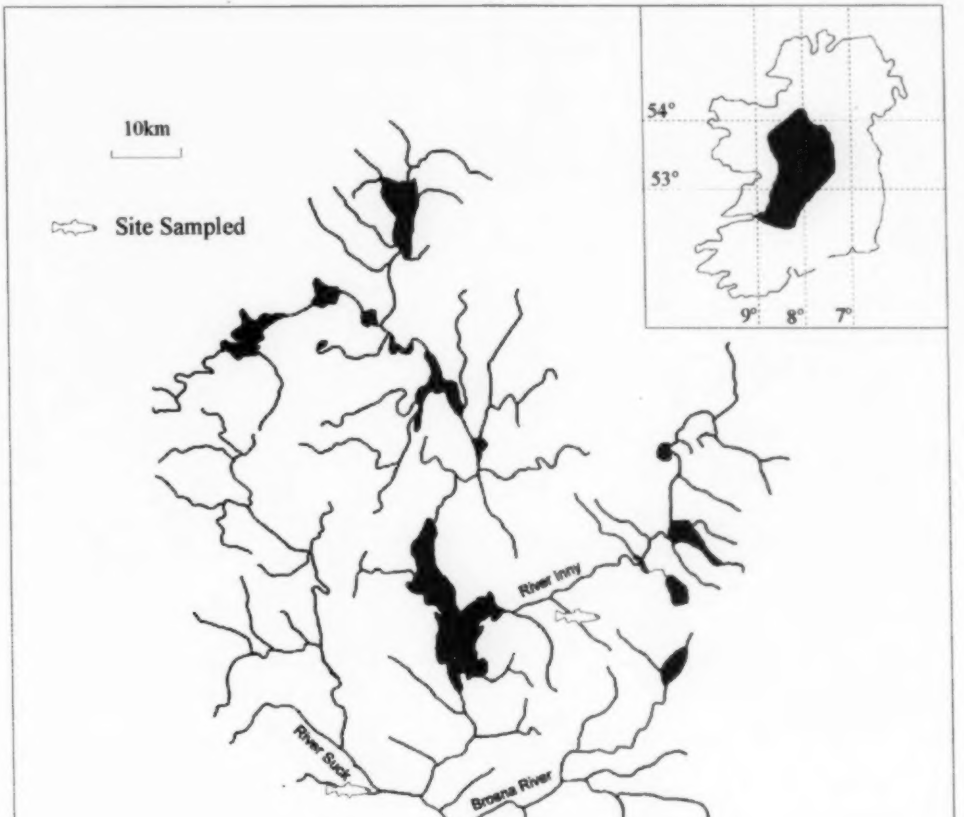

10km
Site Sampled
54°
53°
9°
8°
7°
River Inny
River Suck
Brosna River
Cappagh River
Little Brosna
Nenagh River
Blackwater River
Kilmastulla River
Limerick
Mulkear River
Killaloe
Parteen Regulating Weir
Kilmastulla River
Parteen Hatchery
headrace
Ardnacrusha Generating Station
River Shannon (old channel)
tailrace
Mulkear River
Limerick

Table 1. Observed allelic frequencies for each tributary and the hatchery at the three minisatellite loci screened.

		Hatchery	Mulkear	Blackwater (0+)	Blackwater (1+)	Kilmastulla	Nenagh	Cappagh (1991)	Cappagh (1993)	Little Brosna	Brosna (0+)	Brosna (1+)	Suck	Inny
Ssa-A45/1	*n*	192	102	98	97	86	96	99	81	98	96	99	38	67
	4.4kb	0.000	0.000	0.000	0.005	0.006	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000
	3.1	0.000	0.000	0.010	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000
	2.9	0.565	0.412	0.587	0.497	0.535	0.516	0.490	0.531	0.684	0.594	0.586	0.618	0.627
	2.8	0.013	0.005	0.000	0.010	0.000	0.026	0.040	0.019	0.000	0.005	0.005	0.000	0.015
	2.7	0.102	0.176	0.133	0.124	0.064	0.026	0.071	0.043	0.046	0.078	0.061	0.145	0.104
	2.6	0.216	0.319	0.184	0.263	0.308	0.198	0.338	0.210	0.168	0.266	0.303	0.237	0.179
	2.5	0.000	0.000	0.015	0.005	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.022
	2.1	0.102	0.054	0.061	0.072	0.076	0.234	0.061	0.167	0.061	0.052	0.040	0.000	0.052
	2.0	0.003	0.034	0.010	0.021	0.012	0.000	0.000	0.025	0.041	0.000	0.000	0.000	0.000
Ssa-A45/2/1	*n*	192	101	98	96	86	96	99	81	99	95	98	33	65
	9.1kb	0.013	0.000	0.005	0.000	0.006	0.000	0.026	0.000	0.000	0.021	0.020	0.000	0.000
	8.5	0.263	0.317	0.265	0.250	0.221	0.057	0.209	0.321	0.194	0.232	0.194	0.045	0.154
	8.1	0.016	0.020	0.148	0.094	0.052	0.005	0.051	0.086	0.046	0.032	0.036	0.212	0.008
	7.6	0.013	0.020	0.005	0.000	0.029	0.016	0.010	0.012	0.041	0.005	0.000	0.000	0.062
	6.7–7.1	0.487	0.312	0.276	0.375	0.442	0.625	0.495	0.444	0.561	0.489	0.449	0.727	0.462
	6.1	0.018	0.015	0.005	0.005	0.023	0.000	0.026	0.000	0.000	0.000	0.010	0.000	0.138
	5.5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
	5.3	0.102	0.059	0.041	0.063	0.035	0.042	0.020	0.068	0.051	0.079	0.148	0.015	0.031
	5.2	0.052	0.183	0.199	0.125	0.140	0.203	0.010	0.000	0.087	0.084	0.066	0.000	0.031
	4.7	0.036	0.074	0.051	0.089	0.052	0.052	0.153	0.068	0.015	0.058	0.077	0.000	0.115
Str-A9	*n*	193	103	98	97	86	96	98	81	98	96	99	38	68
	5.4kb	0.101	0.068	0.092	0.119	0.145	0.068	0.025	0.043	0.097	0.099	0.152	0.000	0.081
	4.5	0.158	0.296	0.291	0.237	0.134	0.036	0.152	0.191	0.199	0.193	0.172	0.118	0.140
	4.2	0.386	0.427	0.429	0.423	0.378	0.542	0.535	0.593	0.464	0.422	0.429	0.526	0.449
	3.6	0.018	0.000	0.005	0.000	0.017	0.000	0.000	0.000	0.010	0.005	0.025	0.145	0.007
	3.2	0.062	0.015	0.010	0.021	0.052	0.255	0.010	0.031	0.041	0.047	0.091	0.013	0.132
	2.8	0.057	0.010	0.015	0.021	0.058	0.021	0.000	0.000	0.015	0.005	0.005	0.053	0.096
	2.7	0.036	0.015	0.005	0.041	0.099	0.036	0.141	0.043	0.046	0.099	0.081	0.026	0.007
	2.6	0.181	0.170	0.153	0.139	0.116	0.042	0.136	0.099	0.128	0.130	0.045	0.118	0.088
No. of Observed Alleles		23	22	24	23	23	19	20	19	21	22	22	14	22

and Ferguson 1994) to ensure consistent typing of alleles. Separation was carried out by electrophoresis on 25 cm long 0.77% agarose gels, in 1× TAE buffer at 2V/cm for 24 h. The buffer was mixed twice during the course of the run to prevent it from becoming exhausted. The DNA fragments were then transferred to a Hybond-N™ nylon membrane using standard Southern blotting conditions. Membranes were hybridized sequentially with three minisatellite DNA probes: p*Ssa*-A45/1, p*Ssa*-A45/2 (Taggart et al. 1994), and p*Str*-A9 (Prodöhl et al. 1994*b*). Whereas p*Ssa*-A45/1 and p*Str*-A9 each detect a single minisatellite locus, p*Ssa*-A45/2 reveals distinct "allelic" fragments for two separate loci, termed *Ssa*-A45/2/1 and *Ssa*-A45/2/2 (Taggart et al. 1994, 1995). The former, with alleles ranging in size between 4.5 and 10.0 kilobases (kb), is highly polymorphic. In contrast, *Ssa*-A45/2/2 (allele size range 2.5–3.2 kb) is almost invariant among European Atlantic salmon populations (frequency of most common allele >0.995). A smaller allele predominates in Atlantic salmon of North American origin, providing a highly discriminatory intercontinental stock marker (Taggart et al. 1995).

Statistical analysis

Gene frequency calculations were carried out using the BIOSYS-1 computer program (Swofford and Selander 1981). Genotypic frequencies were tested for conformance with Hardy–Weinberg expectations using a χ^2 test where the significance of the χ^2 value was assessed using a pseudo-probability test (Zaykin and Pudovkin 1993). A similar type program by the same authors enabled a χ^2 homogeneity test to be carried out using all alleles recorded. Where multiple tests were carried out, the sequential Bonferroni correction procedure (Holm 1979) was used to avoid type I statistical errors. Expected heterozygosity estimates together with interlocus variances were calculated and mean heterozygosity estimates were compared with the standard *t*-test as described by Nei (1987). Hierarchical gene diversity analysis of the populations was carried out using NEGST (Chakraborty et al. 1982). Statistical significance of the hierarchical values was assessed using the method described by Workman and Niswander (1970) as modified by Jordan et al. (1992). Mean F_{ST} estimates (jackknifed over loci), together with their 95% confidence intervals (bootstrapped over loci) were calculated using the DIPLOID.FOR program (Weir 1990). While Weir (1990) refers to his estimate of F_{ST} as θ, this discussion will use the original term from Wright (1978) to avoid confusion with the coancestry coefficient of Reynolds et al. (1983) (also θ) which has been used as a pairwise distance in this study. The PHYLIP computer package (Felsenstein 1993) was used to generate a consensus dendrogram based on the allele frequency data. Allele frequencies were bootstrapped over loci (Felsenstein 1985) 100 times using the SEQBOOT program. The resultant bootstrapped frequencies were used to calculate 100 estimates of Reynolds et al. (1983) genetic distance (using the GENDIST program). These genetic distance data were used to generate 100 dendrograms based on the neighbor-joining algorithm. A consensus dendrogram was constructed (using the CONSENSE program) to estimate the confidence to be placed on the nodes of the dendrogram. Data from the Windermere River (Westmoreland, England) (McCubbing and Hartley 1995) and Dee (Aberdeenshire, Scotland) (J. Taggart, unpublished data) were included as outgroups to facilitate interpretation of the dendrogram. Pairwise geographic distances (measured as the shortest distance along rivers between sampling sites) were compared with the corresponding pairwise genetic distances using the Mantel test (Mantel 1967) in the G-STAT computer package (Siegismund 1993).

Results and discussion

All 1252 salmon screened in this study were homozygous for the European diagnostic allele at *Ssa*-A45/2/2. There was no evidence of North American alleles that may have persisted since the historical stocking of smolts derived from ova of Canadian origin. Each of the other three loci detected were polymorphic, with individuals resolving either one or two bands, corresponding to homozygote and heterozygote genotypes, respectively. Combining samples from all tributaries, 9, 10, and 8 alleles were scored at the three polymorphic loci: *Ssa*-A45/1, *Ssa*-A45/2/1, and *Str*-A9, respectively. The ranges of allele sizes were 1.9–4.5 kb for *Ssa*-A45/1, 4.8–9.2 kb for *Ssa*-A45/2/1, and 2.5–5.4 kb for *Str*-A9. The size difference among alleles segregating at *Ssa*-A45/1 and *Str*-A9 resulted in discrete banding patterns and enabled unambiguous scoring. This was also the case for most alleles at *Ssa*-A45/2/1. However, the near continuous distribution of alleles between 6.7 and 7.1 kb in size at this locus prevented consistent typing in this region. These were binned and scored as a single isoallelic class (see Table 1). No "private" alleles (sensu Slatkin 1985) were detected at frequencies higher than 0.005 and only one was detected at this frequency at the *Ssa*-A45/2/1 locus.

While ova from various non-native sources (including 120 000 Canadian ova in 1967) were used in the early years of the enhancement programme, the scale of the operation was then greatly increased and focused on using wild Mulkear fish as brood stock. The Mulkear tributary has a large effective population size. Its catchment area is 646 km^2 and, in terms of juvenile salmon production, is among the top three salmon-producing catchments in Ireland (M. O'Grady, Central Fisheries Board, personal communication). The number of salmon entering the Mulkear each year is also believed to the high, probably in the region of 10 000 fish. In more recent years, brood stock have been collected from adult upstream migrants ascending at Parteen regulating weir. As large numbers of brood stock are used for the enhancement stock ($n > 500$), it is not surprising to note that the hatchery stock has comparable levels of genetic variability to those found in the wild populations of the Shannon and indeed those of the Windermere (Westmoreland, England), Dee (Aberdeenshire, Scotland) and Munster Blackwater (C. Stone, School of Biology and Biochemistry, The Queen's University of Belfast, unpublished data). Therefore, the current enhancement stock does not appear to have compromised genetic variability. However, the absence from any of the 1252 fish screened of distinctive Canadian alleles detectable at *Ssa*-45/2/2 questions the success of the earlier restocking efforts and the wisdom of using non-native stocks. Anecdotal evidence suggests that the smolts released from those ova were in good condition and that the methods applied at the time were effective in the production of adult returns. It is, therefore, notable that, from 120 000 of a total 22.9 million fish stocked into the surveyed tributaries up to 1990, there was no evidence of Canadian alleles in the present survey.

Mean heterozygosity values ranged from 0.553 in the River Suck, to 0.720 in the Mulkear River (Table 2). Six deviations of genotypic frequencies from Hardy–Weinberg proportions were detected ($p < 0.05$) of 39 tests carried out. However, using the sequential Bonferroni correction procedure for multiple tests (Holm 1979) and taking $\alpha = 0.05$ (where α is the experimentwise error rate), none of the tests was significant. Therefore the deviations from Hardy–Weinberg proportions detected were no greater than would have been expected by chance alone.

Table 2. Estimates of intralocus heterozygosities (with SEs given in parentheses) for three minisatellite loci for nine of the tributary populations and the hatchery.

	Ssa-A45/1	*Ssa*-A45/2/1	*Str*-A9	Average heterozygosity
Hatchery	0.615 (0.021)	0.680 (0.018)	0.782 (0.013)	0.692 (0.049)
Mulkear	0.697 (0.017)	0.762 (0.015)	0.699 (0.018)	0.720 (0.021)
Blackwater	0.645 (0.019)	0.777 (0.009)	0.735 (0.012)	0.719 (0.039)
Kilmastulla	0.612 (0.027)	0.732 (0.025)	0.793 (0.020)	0.713 (0.053)
Nenagh	0.642 (0.024)	0.565 (0.034)	0.635 (0.029)	0.614 (0.025)
Cappagh	0.649 (0.018)	0.692 (0.018)	0.633 (0.023)	0.658 (0.017)
Little Brosna	0.499 (0.038)	0.622 (0.033)	0.719 (0.025)	0.613 (0.064)
Brosna	0.565 (0.020)	0.712 (0.018)	0.751 (0.016)	0.676 (0.057)
Suck	0.548 (0.047)	0.431 (0.062)	0.680 (0.048)	0.553 (0.072)
Inny	0.565 (0.043)	0.730 (0.031)	0.743 (0.029)	0.679 (0.057)

Table 3. Absolute and relative gene diversities calculated from a hierarchical analysis of gene diversity.

	Relative gene diversity (%)		Absolute gene diversity		
Locus	Within samples	Among year-classes	Among tributaries	Within samples	Total
Ssa-A45/1	97.49	0.25	2.26	0.602	0.618
Ssa-A45/2/1	94.87	0.19	4.94	0.667	0.702
Str-A9	96.92	0.12	2.96	0.697	0.719
Mean	96.39	0.18	3.43	0.655	0.680
SE	0.80	0.04	0.80	0.028	0.031

Nine χ^2 pairwise homogeneity tests were carried out to assess temporal stability at each of the loci in the Blackwater, Cappagh, and Brosna tributaries. Following the sequential Bonferroni correction for multiple tests ($\alpha = 0.05$), *Ssa*-A45/1, *Ssa*-A45/2/1, and *Str*-A9 revealed heterogeneity ($p = 0.001$, $p < 0.001$, and $p = 0.015$, respectively) between the two year-classes collected from the Cappagh River. No significant heterogeneity was found between year-classes in either the Blackwater or Brosna tributaries. Bootstrapped F_{ST} values were consistent with the χ^2 analysis and also indicated a significant difference between the genetic compositions of the two year-classes from the Cappagh River (F_{ST} 95% confidence intervals: 0.0078 and 0.0188), while the lower 95% confidence interval was less than zero in pairwise comparisons of year-classes from both of the other two tributaries tested. Significant differences between the genetic compositions of different year-classes as detected in the Cappagh River are consistent with the occurrence of high levels of genetic drift. As with spatial separation, when effective population sizes are small, allelic frequencies between different year classes might be expected to vary due to stochastic factors (Waples 1989). It is significant that of the three rivers examined, the Brosna, Blackwater, and Cappagh, only in the Cappagh where adult spawning escapement is known to be low (M. O'Grady, Central Fisheries Board, Dublin, Ireland, personal communication) was temporal instability evident. While temporal sampling of the Blackwater and Brosna differed to that of the Cappagh, in having samples of consecutive year-classes instead of a 1 year interval between year-classes as in the Cappagh, it is unlikely that this factor would have prevented the detection of temporal instability in the former two tributaries had it been present. Samples from the different year-classes of the Cappagh River were pooled for the estimation of genetic distances, based on the assumption that the differences among year-classes were due to the effects of genetic drift (Waples, 1989); therefore, pooled year-classes were likely to provide the best characterisation of that tributary.

The phenomenon of genetic drift has been well documented in Atlantic salmon, where many hatcheries have in the past used insufficient numbers of brood stock to found the hatchery stock, resulting in far greater differences in the genetic compositions of the hatchery stocks than those of the wild populations from which they were derived (e.g., Cross and King 1983; McElligott et al. 1987; Ståhl 1987; Verspoor 1988). Chakraborty and Nei (1977) demonstrated that, after a bottleneck, genetic distance rapidly increases in the first few subsequent generations. Evidence from studies of human Polynesian populations (Flint et al. 1989) confirm that minisatellite loci can detect bottlenecks, that are not detectable by studies employing allozyme and mitochondrial DNA techniques. There is little evidence in this study, however, of any substantial reductions in either allelic diversity or heterozygosity. Wright (1931) points out that random drift, exacerbated after a bottleneck, can result in reduced allelic diversity, but it can also result in an increase in the frequencies of formerly rare alleles. It may be, therefore, that the short-term nature of the suspected bottleneck effects in the upper Shannon system, have had insufficient time to cause any significant reduction in allelic diversity or heterozygosity. There is also the possibility that, through the early maturation of some of the salmon in the form of precocious parr, the effective population size is not reduced to the extent that might be expected based on the level of adult escapment (Jordan and Youngson 1992).

Hierarchical analysis of variance (Table 3) revealed that 96.4% of the total genetic diversity was within samples. Variation among tributaries accounted for a further 3.4% ($p < 0.001$), while the among-year-class component contributed

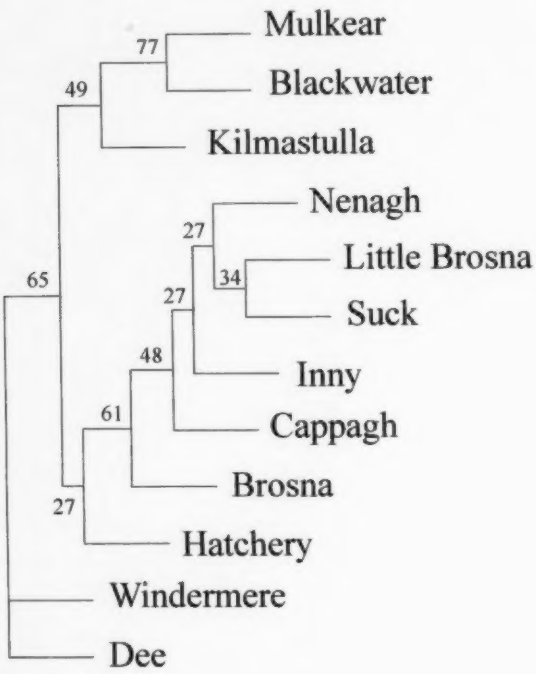
Table 4. Mean estimates of F_{ST} between pairs of tributaries jackknifed over loci, together with upper and lower 95% confidence intervals calculated by bootstrapping over loci.

	Mulkear	Blackwater	Kilmastulla	Nenagh	Cappagh	Little Brosna	Brosna	Suck	Inny
Hatchery									
Estimate	0.0251	0.0155	0.0055	0.0484	0.0154	0.0095	0.0052	0.0465	0.0119
Lower 95% CI	0.0144	0.0004	0.0029	0.0172	0.0050	0.0049	0.0010	0.0055	0.0016
Upper 95% CI	0.0333	0.0330	0.0085	0.0623	0.0306	0.0153	0.0087	0.1007	0.0240
Mulkear									
Estimate		0.0067	0.0195	0.0873	0.0295	0.0486	0.0265	0.0958	0.0433
Lower 95% CI		–0.0016	0.0147	0.0536	0.0176	0.0058	0.0179	0.0316	0.0300
Upper 95% CI		0.0166	0.0270	0.1057	0.0398	0.0820	0.0325	0.1861	0.0522
Blackwater									
Estimate			0.0110	0.0702	0.0220	0.0268	0.0148	0.0694	0.0271
Lower 95% CI			0.0055	0.0274	0.0064	0.0012	0.0069	0.0033	0.0049
Upper 95% CI			0.0163	0.0887	0.0313	0.0522	0.0251	0.1386	0.0469
Kilmastulla									
Estimate				0.0441	0.0158	0.0136	0.0024	0.0518	0.0148
Lower 95% CI				0.0264	–0.0013	0.0014	–0.0001	0.0120	0.0106
Upper 95% CI				0.0580	0.0329	0.0274	0.0046	0.1023	0.0181
Nenagh									
Estimate					0.0517	0.0439	0.0454	0.0655	0.0412
Lower 95% CI					0.0148	0.0255	0.0380	0.0563	0.0251
Upper 95% CI					0.0776	0.0559	0.0485	0.0796	0.0630
Cappagh									
Estimate						0.0214	0.0220	0.0467	0.0223
Lower 95% CI						0.0095	0.0064	0.0184	0.0186
Upper 95% CI						0.0346	0.0313	0.0940	0.0294
Little Brosna									
Estimate							0.0090	0.0327	0.0136
Lower 95% CI							0.0010	0.0143	0.0014
Upper 95% CI							0.0183	0.0643	0.0274
Brosna									
Estimate								0.0456	0.0132
Lower 95% CI								0.0024	0.0074
Upper 95% CI								0.0909	0.0200
Suck									
Estimate									0.0459
Lower 95% CI									–0.0028
Upper 95% CI									0.1045

only 0.2% (not significant) of the total genetic diversity. This figure of 3.4% is comparable with that found in other studies. In studies of the undisturbed Windermere (McCubbing and Hartley 1995) and Dee (Aberdeen) (J. Taggart, unpublished data) systems, the same minisatellite loci as those screened in the present study reveal that 5.0 and 0.7%, respectively, of the total gene diversity is due to differences among tributaries. Ståhl (1987) reported that 3.6% of the allozyme genetic diversity in Atlantic salmon from throughout the range was due to variation between samples within drainages. McElligott and Cross (1991) attributed 3.4% to variation among sites within the Munster Blackwater River system in Ireland, while Jordan et al. (1992) from a study of Atlantic salmon populations from throughout Scotland, found that variation among tributaries of the Tweed accounted for only 0.7% of the total genetic diversity (only 1.6% of the total was due to differences among rivers). Superficially, therefore, it would appear that the among-tributary genetic diversity of the Shannon lies within the ''normal'' range which exists in undisturbed systems. However, in the case of the Shannon, the among-tributary component of genetic diversity is the product of the homogenizing effect of gene flow and genetic differentiation, caused by random drift. Thus, the historic level of genetic differentiation among tributary populations may be being masked by an exacerbated level of gene flow due to the enhancement programme.

Pairwise F_{ST} estimates based on Weir's (1990) program allow an accurate evaluation of genetic differentiation estimates, by providing 95% confidence intervals (Table 4). Where there is a large difference between the 95% confidence intervals, it is likely that random genetic drift is involved, in which the loci yield different F_{ST} values because of the stochastic nature of this type of differentiation (Weir 1990). From the pairwise F_{ST} estimates listed in Table 4, it is

Fig. 2. Neighbor-joining dendrogram based on Reynolds et al. (1983) coancestry coefficient. Values on the nodes represent the number of times that the particular node occurred in 100 dendrograms generated from genetic distances calculated by bootstrapping gene frequencies.



interesting to note that the salmon from the Mulkear and Blackwater tributaries are not significantly different. Both these tributaries meet the main channel below the dam. It is also notable that the Kilmastulla and Brosna tributaries are not significantly different from each other and that pairwise comparisons of each with the hatchery stock reveal low F_{ST} values. In contrast, rivers such as the Nenagh and Suck have relatively high intersample F_{ST} values, with large fluctuations between the estimates from the different loci, as indicated by the magnitude of the differences between the upper and lower 95% confidence intervals. This is quite evident in the F_{ST} estimates for the River Suck and to a lesser extent for the Nenagh and Cappagh rivers. The similarity between the Kilmastulla and Brosna tributaries and the hatchery stock may reflect successful restocking of these tributaries. The Kilmastulla has only recently been recolonized by Atlantic salmon following the recovery of this river from extensive silver mining operations during the 1970s (Bracken et al. 1989), and the contribution of the enhancement programme to this recolonization is likely to have been substantial. Likewise, the Brosna, which has a very favourable salmonid habitat, has received a considerable number of juvenile salmon from the enhancement programme over the years, and so its similarity with the hatchery is not surprising.

Reynolds et al. (1983) coancestry coefficient was chosen as an appropriate genetic distance for determining the genetic relationships among the salmon from the different tributaries. This method neither assumes equal rates of divergence across populations nor constant rates of evolution over loci. The Mulkear, Blackwater, and Kilmastulla populations group together in the neighbor-joining dendrogram (Fig. 2), which is noteworthy as these are the only tributaries to enter the main channel below the dam. While the Mulkear and Blackwater were not significantly different based on the F_{ST} value (lower 95% CI = –0.0016), χ^2 analysis indicated heterogeneity at two of the loci ($p < 0.01$). That these two tributaries are linked together in 77 of 100 iterations offers a crude form of calibration for the rest of the dendrogram. The relatively low values of consensus for the nodes linking the tributaries occurring above the dam in the dendrogram, suggests that the current configuration of this part of the dendrogram should be regarded with extreme caution.

Despite the relatively low consensus values for many of the nodes, the consensus neighbor-joining dendrogram does make biological sense. Neither the Dee nor the Windermere rivers associated closely to any of the Shannon tributaries; this is reasonable since they are both outside of the Shannon system (with relatively distant geographical separation). The splitting of the Shannon into above and below the dam groups is also reasonable. While the hatchery is positioned with the tributaries above the dam, the low consensus value (27/100) suggests that there may be contradictions among loci as to its most appropriate position. This is understandable as the upper tributaries are likely to have a large component of hatchery derived genotypes (from earlier restocking efforts), while the Mulkear was the original source population for the hatchery stock. It is not surprising, therefore, given the complexity of the Shannon population structure, which is likely to lead to a considerable number of homoplasies, that a dendrogram with greater consensus has not emerged.

Geographic distances among sampling sites were not significantly correlated with Reynolds et al. (1983) coancestry coefficient for any of the three loci or for the three loci combined. The absence of a correlation between geographical and genetic distances might be interpreted as being consistent with the hypothesis that random genetic drift is the most important evolutionary force shaping the population genetic structure of salmon populations in the river. However, geographical distances measured within a river system assume that the migration distances experienced by a salmon are proportional to the length of the river. This may not be the case because of the characteristic heterogeneity of freshwater habitats, where differential flow rates, obstacles, and predators make the estimation of the true geographic distance extremely difficult. Where the tributaries are relatively long, it is also possible that the nursery areas from which the salmon were collected could be a considerable distance from the spawning sites, contributing an additional source of error when the geographic distances are measured between sampling sites.

General discussion

The use of single-locus minisatellite DNA data to analyse the population genetic structure of salmon in the Shannon system demonstrates that this relatively novel technique has considerable potential for discriminating among Atlantic salmon populations. The number of alleles and heterozygosity detected by three of the loci far exceeds that revealed by either allozyme electrophoresis or mitochondrial DNA analysis for similar type studies reported in the literature. In the context of the current study, *Ssa*-A45/2/2 enabled an evaluation of the success rate of the use of that non-native strain in the Shannon enhancement programme. The absence of Canadian alleles at that locus in any of the 1252 salmon screened suggests that a similar type evaluation is required in other European river systems where enhancement programmes have utilized Canadian strains in the past.

As with most studies of this type, extrapolation from the present picture of historic changes to the population structure, and determination of the forces that moulded those changes is extremely difficult. Yet the combination of temporal instability of allele frequencies in one of the two tributaries analysed from the upper system, combined with large differences between the 95% confidence intervals for F_{ST} estimates and low values for the nodes of the bootstrapped dendrogram, suggests that genetic drift has been the primary force shaping the current population structure of the upper system.

Based on the recent history of this river system, two major forces are expected to shape the population structure: first, gene flow, substantially increased by the enhancement programme, having the effect of homogenizing the genetic compositions of the salmon populations throughout the system and secondly, genetic drift caused by population bottlenecks. The latter may be attributed to difficulties associated with smolt downstream passage and adult upstream passage at Ardnacrusha generating station and at Parteen regulating weir, resulting in small effective population sizes in the upper parts of the system, leading to stochastic drift of allelic frequencies and a high degree of genetic differentiation between salmon from neighbouring tributaries.

This study, therefore, demonstrates geographical variation of Atlantic salmon in a disturbed system. The use of single-locus minisatellite DNA analysis provides a valuable means of assessing population structures even where populations have only recently diverged; it is thus reasonable to assume that this analysis approach would be even more powerful when applied to systems which have not been subjected to such a degree of human interference. Information of this nature is of the utmost importance when management of a fishery seeks to maximize the conservation of genetic diversity with available resources.

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Uptake of nitrite, nitrate, and bromide in rainbow trout, *Oncorhynchus mykiss*: effects on ionic balance

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Abstract: Effects of uptake into blood plasma of NO_2^-, NO_3^-, and Br^- from 1 mM ambient concentrations were studied in rainbow trout (*Oncorhynchus mykiss*). Nitrite and bromide were concentrated in plasma, competing for the branchial chloride uptake mechanism. Nitrate appeared to be taken up passively, with plasma concentrations remaining below ambient $[NO_3^-]$ after 8 days exposure. This limited uptake appeared central to the low toxicity of NO_3^-, and did not measurably influence electrolyte balance or haematology. Plasma $[Br^-]$ increased to 51 mM during 14 days, which was paralleled by a 1:1 stoichiometrical decrease in plasma $[Cl^-]$. This was the only detected effect of Br^- exposure and was tolerated without mortality. Nitrite-exposed trout fell into two distinct groups. Trout dying before 2 days of NO_2^- exposure quickly developed methaemoglobinaemia; high plasma $[NO_2^-]$, [lactate], and $[K^+]$; and low $[Cl^-]$. In trout surviving up to 4 days, plasma $[NO_2^-]$ and methaemoglobin rose more slowly, plasma $[Cl^-]$ decreased less, and extracellular lactate and potassium levels were not significantly elevated. In both groups, plasma $[NO_3^-]$ rose to values comparable with plasma $[NO_2^-]$ (about 3 mM), reflecting an internal conversion of nitrite to nitrate. Nitrite-exposure significantly decreased skeletal muscle $[K^+]$, whereas no significant changes were observed in cardiac muscle.

Résumé : Nous avons étudié chez la truite arc-en-ciel (*Oncorhynchus mykiss*) les effets que produit l'entrée dans le plasma sanguin du NO_2^- du NO_3^- et du Br^- à partir de concentrations ambiantes de 1 mM. Le nitrite et le brome se concentrent dans le plasma et concurrencent le chlore pour accaparer le mécanisme d'absorption du chlore au niveau des branchies. Le nitrate paraît être absorbé passivement, sa concentration plasmatique demeurant inférieure à la concentration ambiante au bout de 8 jours d'exposition. Son absorption limitée pourrait expliquer la faible toxicité du NO_3^-; en outre, elle n'a pas modifié l'équilibre électrolytique ou hématologique des sujets d'expérience. La $[Br^-]$ plasmatique s'est élevée jusqu'à atteindre 51 mM au bout de 14 jours; à l'inverse, la $[Cl^-]$ plasmatique s'est abaissée dans un rapport stoechiométrique de 1 à 1. C'est le seul effet détecté de l'exposition au Br^-, et il a été toléré sans mortalité. Les truites exposées au nitrite se divisaient en deux groupes. Celles qui mouraient avant 2 jours d'exposition au NO_2^- étaient vite atteintes de méthémoglobinémie; en outre, la $[NO_2^-]$, la [lactate] et la $[K^+]$ étaient élevées tandis que la $[Cl^-]$ était basse. Chez les truites qui survivaient jusqu'à quatre jours, la $[NO_2^-]$ plasmatique et le taux de méthémoglobine s'élevaient plus lentement, la $[Cl^-]$ plasmatique s'abaissait moins et les taux de potassium et de lactate extracellulaire ne s'étaient pas accrus de manière importante. Dans les deux groupes, la $[NO_3^-]$ plasmatique s'est élevée jusqu'à des valeurs comparables à celles du $[NO_2^-]$ plasmatique (environ 3 mM); cela témoigne d'une conversion du nitrite en nitrate dans l'organisme. L'exposition au nitrite provoque un abaissement considérable de la $[K^+]$ dans les muscles striés, mais aucun effet marqué n'a été observé sur le muscle cardiaque.
[Traduit par la Rédaction]

Introduction

Maintenance of osmotic homeostasis in freshwater fish depends on an active uptake of ions across the gills that balances urinary and passive branchial ion losses. Branchial transport mechanisms for monovalent anions have evolved to handle physiologically important chloride and bicarbonate ions. The selectivity for these anions is not perfect, however. Other anions that are present at variable concentrations in the water can have an affinity for the uptake mechanism. One such anion is nitrite, which competes with chloride for the active chloride uptake mechanism in the gills (Williams and Eddy 1986), resulting in accumulation of nitrite in the extracellular fluid when ambient nitrite levels are elevated. Uptake of nitrite disturbs the electrolyte balance by inducing a net chloride loss and an extracellular hyperkalaemia (rise in plasma $[K^+]$) (Jensen et al. 1987). Entry of nitrite into the red blood cells (RBCs) induces oxidation of haemoglobin (Hb) to methaemoglobin (metHb) and, in carp, activation of KCl cotransport, resulting in RBC K^+ loss and RBC shrinkage (Jensen 1990*a*, 1992). The efflux of K^+ from the RBCs contributes to the extracellular hyperkaelemia. The potential contribution to the hyperkalaemia from loss of K^+ from muscle tissue, as seen in crayfish (Jensen 1990*b*), has not yet been investigated in teleosts.

Problems with elevated ambient nitrite levels are often transient and limited to certain hypoxic habitats, habitats receiving nitrogenous pollutants, and recirculated aquaculture

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systems. High ambient nitrate levels (reaching the millimolar range), in contrast, occur in numerous aquatic habitats resulting from agricultural use of fertilizers (Bouchard et al. 1992). Nitrate is relatively nontoxic to fish (Westin 1974; Colt and Tchobanoglous 1976), but the potential uptake of nitrate has not been investigated. It is pertinent to investigate whether NO_3^- is taken up via the same route as NO_2^-.

Other anions with potential affinity for branchial anion transport mechanisms include halides such as bromide. Methyl bromide is used on a large scale as a soil fumigant in intensive horticulture in the Netherlands, where bromide (Br^-) in the drainage water can reach concentrations of 0.9 mM (Wegman et al. 1981; Wegman et al. 1983). The toxicity of Br^- to fish is low (Alexander et al. 1981; Canton et al. 1983; Wester et al. 1988; Yarzhombek et al. 1991), but the uptake of Br^- has not been investigated.

The present study investigated the uptake of NO_2^-, NO_3^-, and Br^- from the environmental water, and the possible effects of these anions on the extracellular ionic balance and on skeletal and cardiac muscle tissue potassium and water content in rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Experimental animals

Rainbow trout were obtained from a local hatchery. Two different size-classes of fish were used in the experiments. Larger fish (weight 336 ± 58 g, mean ± SD, $N = 33$) were used in cannulation experiments, and smaller ones (weight 119 ± 27 g, $N = 93$) were used uncannulated. The fish were acclimated to aerated Odense tap water for at least one month before experiments. In this pre-experimental period, the larger fish were kept in a tank filled with 7000 L of water, and the smaller ones in a 500-L tank. The water was renewed by a continuous inflow of tap water. The fish were fed with commercial trout food pellets until 3 days before experiments, and were subjected to a 12 h light: 12 h dark photoperiod.

Series I: uptake and effects of nitrite, nitrate, and bromide in individual cannulated fish

The fish were anaesthetized in a 1 : 10 000 solution of MS 222 (3-aminobenzoic acid ethyl ester, Sigma) for approximately 4 min and then catheterized in the dorsal aorta with polyethylene tubing (i.d. 0.58 mm, o.d. 0.96 mm) (Soivio et al. 1975), while irrigating the gills with aerated water containing MS 222 (1:20 000). After the operation (< 2 min), the fish were individually placed in 50-L glass aquaria with 34 L of tap water at 15°C, and allowed to recover for 48 h. The aquaria were covered with polystyrene sheets on all but one side to minimize disturbances from the surroundings. The water was aerated ($Po_2 > 135$ mmHg), and 20 L was renewed daily throughout the experimental period.

After withdrawal of a control blood sample, either $NaNO_2$, $NaNO_3$, or NaBr (Merck, pro analysis) was added to the water to a concentration of 1 mM. This was defined as time zero in the experiments. Blood samples (0.6 mL) were withdrawn at 0 and 3 h, and subsequently once a day up to (*i*) 14 days in control fish maintained in tap water ($N = 8$); (*ii*) 4 days in nitrite-exposed fish ($N = 13$); (*iii*) 8 days in nitrate-exposed fish ($N = 5$); and (*iv*) 14 days in bromide-exposed fish ($N = 7$).

Series II: exposure effects on tissue K^+ and water contents in non-catheterized fish

It was not feasible to obtain muscle and cardiac tissue samples in series I, which involved repetitive blood sampling from individual fish. Exposure effects on tissue K^+ and water content were investigated in series II, using smaller fish (since these were to be sampled only once). Six fish were placed in each 50-L aquarium and exposed to either control water or to 1 mM concentrations of $NaNO_2$, $NaNO_3$, or NaBr for a predetermined period. At the end of this period, all six fish in each aquarium were sampled. Fish were quickly netted and stunned with a blow to the head. A blood sample (0.8–1 mL) for comparison with the results from series I was drawn from the caudal vessels into a heparinized syringe by mild suction. Then the heart and a piece of caudal muscle were dissected out, and the caudal muscle was separated from the skin. The beating heart was placed in a bath with physiological saline for approximately 1 min to rinse the blood from it. Half the heart and a piece of the muscle tissue were frozen (−20°C) instantly for later analysis of K^+ content. The other half of the heart and a large piece of the caudal muscle (2–4 g) were dried at 105°C for 24 h to determine tissue water content from the difference in wet and dry masses. Control fish were sampled at 1, 4, 5, 7, 8, and 14 days, nitrite-exposed fish, at 1 and 4 days; nitrate-exposed fish, at 1, 5, and 8 days; and bromide-exposed fish, at 1, 7, and 14 days. Absolute concentrations and time-dependent changes in plasma ions were similar in series I and series II fish, revealing that the fish responded alike to the treatments, and that differences related to fish size or experimental design (presence of one vs. six fish in the aquaria during the exposures) were absent. Only the supplementary information regarding tissue K^+ and water content, aimed at with series II, is presented from this series.

In both experimental series, water temperature was 15.0 ± 0.2°C ($N = 254$) and water pH was about 8.3. The measured ionic composition of control water was (mM, means of 35–42 measurements): $[Cl^-] = 1.39$, $[Br^-] = 0.002$, $[NO_2^-] = 0.003$, $[NO_3^-] = 0.028$, $[SO_4^{2-}] = 1.50$, $[Na^+] = 1.46$, $[K^+] = 0.16$, $[Mg^{2+}] = 0.64$, $[Ca^{2+}] = 3.03$. During the exposures the measured concentrations of NO_2^-, NO_3^-, and Br^- (in millimolar, with N given in parentheses) were as follows: $[NO_2^-] = 1.01 \pm 0.02$ (67), $[NO_3^-] = 1.01 \pm 0.026$ (20), and $[Br^-] = 1.00 \pm 0.05$ (55).

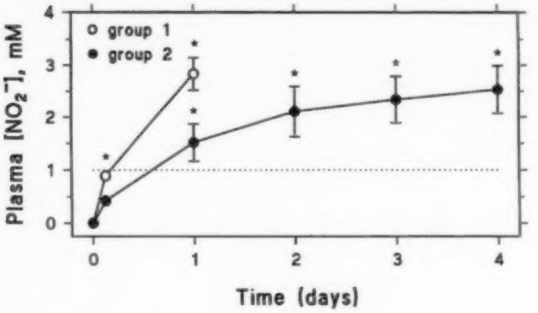
Measurements

Haematocrit was determined by centrifugation (2 min at 15 000 × *g*) and plasma and water nitrite concentrations, as well as blood total haemoglobin (Hb) and methaemoglobin (metHb), were measured by spectrophotometric (Milton Roy Spectronic 1201) methods (cf. Jensen, 1992). The concentrations of anions (Cl^-, Br^-, NO_3^-, HPO_4^{2-}, SO_4^{2-}) and cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) in plasma and water were measured on an ion-exchange chromatograph (Dionex Series 4500i), using anion and cation exchange columns, respectively, and a conductivity detector. Inorganic phosphate will be referred to as HPO_4^{2-}, since more than 80% of the phosphate will be in this form at physiological pH. Plasma lactate was assessed spectrophotometrically by the Boehringer–Mannheim lactate dehydrogenase method and plasma osmolality was measured on a Gonotec Osmomat 030.

Skeletal muscle tissue (about 1 g) was extracted with 5.0 mL 0.1 M HNO_3 for 72 h and then homogenized. The homogenate was centrifuged for 15 min at 3020 × *g*, and the supernatant was stored at −20°C until analysis. The heart tissue (approximately 70 mg) was extracted with a stronger acid (1.0 ml 0.2 M HNO_3). After 72 h, the tissue was placed in an oven at 60°C for 4 days, whereupon it was so tender that it could be homogenized with a plastic spatula. The homogenate was centrifuged for 5 min at 13 490 × *g*, and the supernatant was stored at −20°C. The potassium content of the tissues was measured by atomic absorption spectrophotometry (Perkin-Elmer 2380).

The statistical significance of changes was evaluated by analysis of variance (ANOVA) for repeated measures followed by the Tukey test in experiments on catheterized fish, and by completely randomized ANOVA followed by the Tukey test in experiments on non-catheterized fish. A completely randomized ANOVA was also used

Fig. 1. Time-dependent changes in plasma nitrite concentration in catheterized rainbow trout exposed to 1 mM ambient nitrite (broken line). Asterisks indicate a significant ($P < 0.05$) difference from the time zero value. See text for explanation of group 1 and group 2. Values are means ± SEs ($N = 8$ in group 1 and $N = 5$ in group 2).



to evaluate differences between means in two groups (NO_2^--exposed fish). In all cases, $P < 0.05$ was considered a significant difference.

Results

Control fish

Catheterized control fish kept in tap water and sampled for up to 14 days showed a gradual decrease in haematocrit and blood [Hb], which was explained by the repetitive blood sampling. The mean cellular [Hb] was about 4.6 mmol/L RBC and did not change with time. Similarly, the concentrations of Cl^-, Br^-, SO_4^{2-}, Na^+, K^+, Ca^{2+} and Mg^{2+} were unchanged with time. Plasma $[NO_2^-]$ and $[NO_3^-]$ never exceeded 10 and 30 μM, respectively. The only ions that showed significant changes were phosphate (which decreased from about 2.4 mM at day 0 to 1.8 mM at day 14) and lactate (which decreased from 1 mM at day 0 to about 0.5 mM at days 4–10 and then rose to 0.8 mM at day 14) (not shown). The tissue K^+ and water content, measured in caudal muscle and cardiac muscle of uncannulated fish, did not change significantly with time up to 14 days.

Nitrite exposure

Rainbow trout exposed to 1 mM ambient nitrite accumulated NO_2^- in the plasma to concentrations above the environmental concentration (Fig. 1). The fish responded differently to nitrite exposure and were divided into two distinct groups: those dying between day 1 and day 2 (group 1, $N = 8$) and those surviving up to day 4 but dying before day 6 (group 2, $N = 5$). The accumulation of nitrite was more rapid in group 1 than in group 2. In group 1, plasma $[NO_2^-]$ reached the ambient concentration after 4 h and 2.8 mM after 1 day. In group 2, plasma $[NO_2^-]$ reached the ambient concentration after 14 h, and 1.5 mM after 1 day (significantly lower than in group 1). After 4 days the plasma $[NO_2^-]$ was 2.5 mM (Fig. 1).

Nitrite-induced oxidation of haemoglobin (Hb) to methaemoglobin (metHb) was significantly faster in group 1 (53% metHb at 1 day) than in group 2 (22% metHb at 1 day). In group 2, the increase in metHb was linear up to day 2, then it levelled off, with a metHb value of 56.2% of the total Hb being reached after 4 days (Fig. 2).

Haematocrit and blood [Hb] decreased in both groups (Fig. 2) at a rate that was larger than in control fish. The red cell Hb concentration did not change significantly, though a tendency for an increase was observed in group 1 (Fig. 2).

Plasma $[NO_3^-]$ increased significantly (Fig. 3) in parallel with the accumulation of nitrite. After 1 day of nitrite exposure, plasma $[NO_3^-]$ was 1.8 mM in group 1 and 1.3 mM in group 2. In group 2, $[NO_3^-]$ reached 3 mM at 4 days. Plasma $[NO_2^-]$ was significantly higher than $[NO_3^-]$ in group 1, whereas there were no significant differences between plasma $[NO_2^-]$ and $[NO_3^-]$ in group 2 (compare Figs. 1 and 3).

Plasma [lactate] increased to very high levels after 1 day of nitrite exposure in group 1 whereas no significant changes occurred over 4 days in group 2 (Fig. 3).

The plasma chloride concentration decreased by 22.2 mM within 1 day in group 1 (Fig. 3). This Cl^- depletion was significantly larger than in group 2, where plasma $[Cl^-]$ declined by 6.2 mM in 4 days (Fig. 3). Similarly, plasma $[Br^-]$ decreased more in group 1 than in group 2 (Fig. 3). The time courses for the bromide decreases resembled those for the chloride decreases (Fig. 3).

Plasma $[HPO_4^{2-}]$ increased significantly (by 1.4 mM) in group 1, whereas only minor changes occurred in group 2 (Fig. 3). The increase in plasma $[HPO_4^{2-}]$ contrasted with the decrease observed in the control group (cf. above). Plasma $[SO_4^{2-}]$ differed slightly between the two groups, but no major exposure effect was observed (Fig. 3).

Plasma $[K^+]$ increased substantially during nitrite exposure in group 1 but not in group 2 (Fig. 4), whereas plasma $[Na^+]$ decreased slightly in group 1 and was unchanged in group 2 (Fig. 4). Plasma $[Mg^{2+}]$ and $[Ca^{2+}]$ increased significantly in group 1 but not in group 2 (Fig. 4).

Only minor and nonsignificant changes were observed in plasma osmolality (not shown).

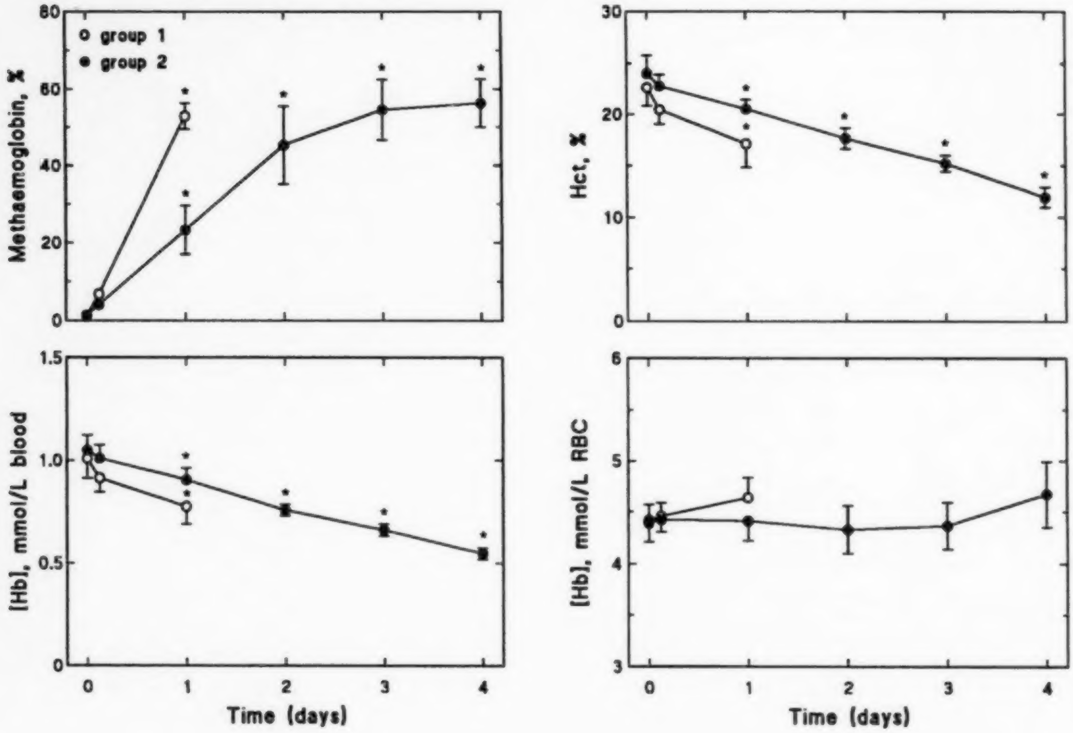
The smaller noncatheterized fish used in series II also fell into two distinct groups when exposed to nitrite, and the effects of nitrite exposure on haematology and ionic parameters were similar to those seen in larger cannulated fish (not shown). The caudal muscle tissue K^+ content showed a large significant decrease in both groups of nitrite-exposed fish, whereas muscle K^+ did not change in control fish (Fig. 5). The muscle K^+ loss in group 2 after 4 days was larger than in group 1 after 1 day of nitrite exposure (Fig. 5). The water content of muscle tissue decreased significantly in nitrite-exposed fish but not in controls (Fig. 5).

In control fish, the K^+ content of heart tissue was lower than the K^+ content of caudal muscle tissue (Fig. 5), suggesting a tissue-specific difference in K^+ content. The measured values of heart K^+ varied widely, and no significant differences were detected between nitrite-exposed fish and control fish (Fig. 5). Also, no significant changes were observed in heart tissue water content (Fig. 5).

Nitrate exposure

Exposure to 1 mM ambient nitrate caused a significant increase in plasma $[NO_3^-]$ (Fig. 6). From day 1 and throughout the experimental period of 8 days, the plasma $[NO_3^-]$ was constant at about 0.2 mM, which was one fifth of the water concentration (Fig. 6). There were no significant changes in plasma osmolality or in the concentrations of Cl^-, Br^-, SO_4^{2-}, lactate, Na^+, K^+, Mg^{2+}, or Ca^{2+}. Plasma $[HPO_4^{2-}]$ and blood

Fig. 2. Time-dependent changes in dorsal aortic methaemoglobin content, arterial haematocrit (Hct), total blood haemoglobin (Hb) concentration, and red cell haemoglobin concentration of nitrite-exposed rainbow trout. Symbols are as in Fig. 1.



[Hb] decreased as in control fish (cf. above). Plasma [NO_2^-] never exceeded 7 μM and metHb stayed below 3% of the total Hb (not illustrated). Muscle and heart tissue K^+ and water content were unaffected by nitrate exposure.

Bromide exposure

Exposure to 1 mM environmental bromide resulted in a considerable uptake of bromide. The plasma [Br^-] increased throughout the 14 days exposure period, reaching a mean value of 51 mM at 14 days (Fig. 7*a*). The accumulation of bromide was paralleled by a significant decrease in the plasma [Cl^-] (Fig. 7*a*). The highly significant linear relationship between [Cl^-] and [Br^-] (Fig. 7*b*) revealed a 1:1 stoichiometrical relationship between the decrease in plasma [Cl^-] and increase in plasma [Br^-]. There were no noteworthy changes in other plasma ions nor in heart and caudal muscle K^+ and water content.

Discussion

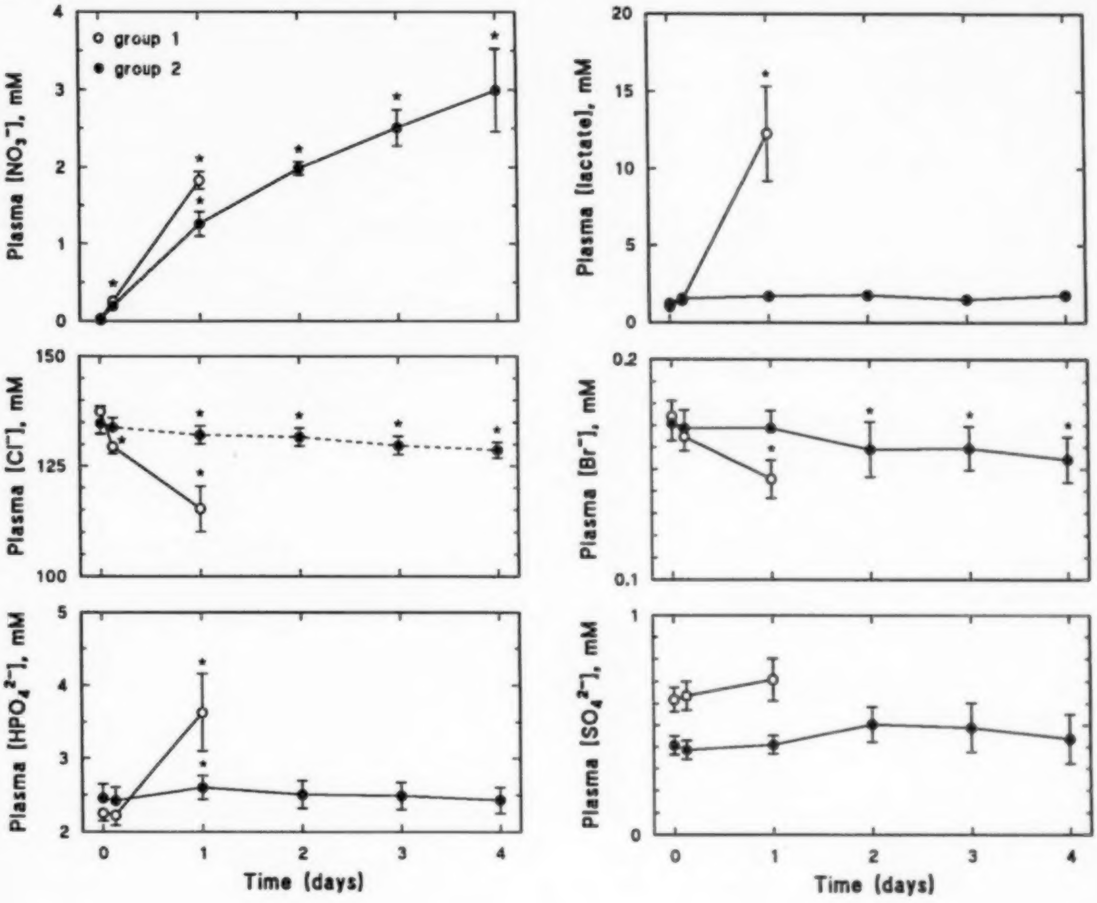
Comparison of nitrite, nitrate, and bromide uptake

Rainbow trout exposed to NO_2^-, NO_3^-, or Br^- at an ambient concentration of 1 mM took up these anions to a strikingly different degree. After 1 day of exposure, the plasma concentrations were 9.2 mM for Br^-, 2.8 versus 1.5 mM (group 1 versus group2) for NO_2^- and 0.2 mM for NO_3^-, revealing the following sequence of uptake rates: $Br^- > NO_2^- > NO_3^-$.

Uptakes of bromide and nitrite were active, being against both a concentration gradient and an electrochemical gradient. Thus, whereas the transepithelial potential (TEP) was about −7 mV (inside negative) in control trout (unpublished observation), the Nernst potential (calculated from plasma and water concentrations of the anions) for bromide reached 97 mV at 14 days of bromide exposure, and the Nernst potential for nitrite was 26 mV in group 1 (at 1 day) and 23 mV in group 2 (at 4 days). For nitrate, in contrast, the Nernst potential was −11 mV (i.e., close to TEP) in the control situation, suggesting that nitrate was passively distributed across the gills. During nitrate exposure the Nernst potential for nitrate was −39 mV. Thus, an electrochemical equilibrium was apparently not reached despite the steady plasma [NO_3^-] of 0.2 mM for days 1–8 (Fig. 6).

Nitrite is known to compete with chloride for the active branchial Cl^- uptake mechanism (Williams and Eddy 1986). From the present data, bromide appears to be taken up via the same mechanism. A partial substitution of Cl^- uptake with Br^- uptake, and a persistent passive Cl^- efflux, would cause the observed Br^- accumulation and Cl^- depletion (Fig 7*a*). The ionic changes during nitrite exposure support this conclusion. Nitrite uptake was associated with decreases in both plasma [Cl^-] and plasma [Br^-] (Fig. 3), as expected if Cl^-, Br^-, and NO_2^- follow the same uptake route. The shared uptake route explains why elevation of ambient [Cl^-] or [Br^-] protects against nitrite toxicity (Eddy et al. 1983). During bromide

Fig. 3. Changes in plasma [NO_3^-], [lactate], [Cl^-], [Br^-], [HPO_4^{2-}], and [SO_4^{2-}] in catheterized nitrite-exposed rainbow trout. Symbols are as in Fig. 1.



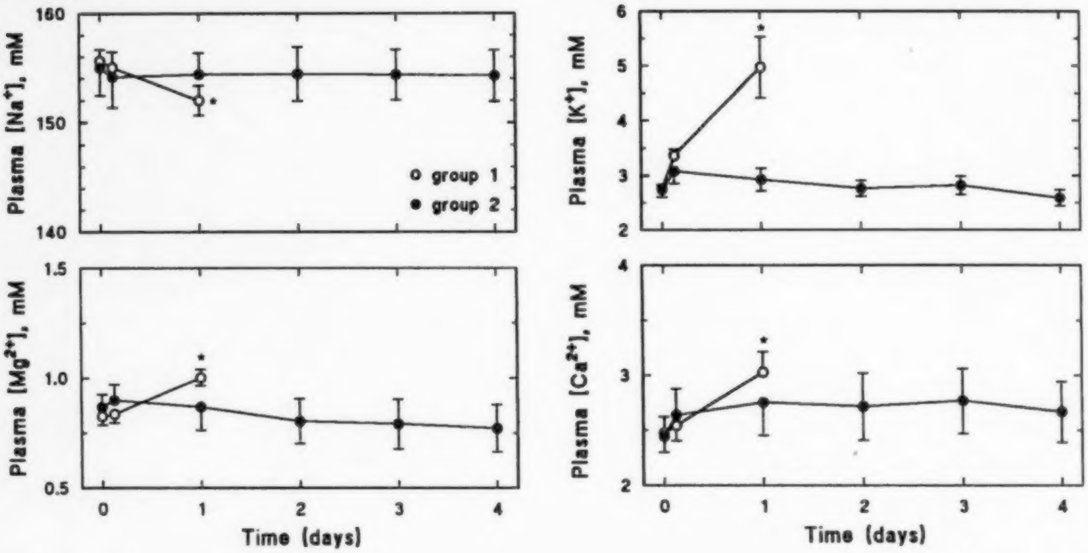
exposure, Br^- accumulation and Cl^- depletion followed a 1:1 stoichiometrical relationship (Fig. 7*b*). The effects of bromide exposure seemed restricted to this gradual substitution of Cl^- with Br^-, which, despite the large magnitude of the changes, was tolerated without mortality or observable effects on well being for the 14-day exposure period.

Nitrate, in contrast to nitrite and bromide, was taken up to a steady plasma concentration which was well below the ambient concentration, revealing an insignificant affinity of nitrate for the active branchial Cl^- uptake mechanism and a primarily passive transport mode. A similar conclusion was reached in freshwater crayfish, *Astacus astacus*, which showed both a slow minor uptake of nitrate and a slow elimination of nitrate, suggesting a low branchial permeability to nitrate as well as a limited urinary loss of nitrate (Jensen 1996). This may also apply to rainbow trout. Urinary loss is, however, not negligible, and the stabilization of plasma [NO_3^-] prior to establisment of electrochemical equilibrium (cf. above) may reflect a quasi-steady balance between passive branchial influx and removal of nitrate from the extracellular compartment via urinary loss and entry into intracellular compartments. The discrimination of the branchial Cl^- uptake mechanism against NO_3^- in both rainbow trout (this study) and crayfish (Jensen 1996) cannot be explained on the basis of ionic size (the ionic radii of Cl^- and NO_3^- are the same) and must depend on other (e.g., steric) factors. The limited uptake and absence of major physiological effects of nitrate in trout and crayfish appears central to the generally low toxicity of nitrate in aquatic animals.

Nitrite-induced effects

The trout fell into two distinct groups with different NO_2^- uptake rates (Fig. 1). Earlier studies on nitrite-exposed rainbow trout have made a similar distinction between relatively tolerant and relatively intolerant specimens (Margiocco et al. 1983; Arillo et al. 1984; Williams and Eddy 1988). Since Cl^- uptake and the fractional area of gill chloride cells are linearily correlated (Perry et al. 1992), one possible explanation of different NO_2^- uptake rates would be intraspecific differences in the number and (or) exposed area of chloride cells, serving to compensate for intraspecific differences in passive anion efflux. Given that bromide shares the same uptake route

Fig. 4. Changes in plasma concentrations of sodium, potassium, magnesium, and calcium in catheterized nitrite-exposed rainbow trout. Symbols are as in Fig. 1.



as chloride and nitrite, this hypothesis suggests intraspecific differences in bromide uptake as well. A distinct intraspecific variation was, however, not observed for bromide accumulation (Fig. 7*a*). However, possible intraspecific differences in chloride cell proliferation during nitrite exposure (Gaino et al. 1984) and in the relative affinity of anions for uptake sites may complicate any attempt to draw a direct conclusion from comparison of NO_2^- and Br^- accumulation. More research is needed to give a definite explanation of the grouping of trout in response to nitrite exposure.

The rapid nitrite accumulation in group 1 was associated with a more rapid developing methaemoglobinaemia than seen in group 2. Red cell KCl efflux and RBC shrinkage, which are prominent effects of nitrite-induced methaemoglobinaemia in carp (*Cyprinus carpio*) (Jensen et al. 1987; Jensen 1990*a*), appeared minor in trout, judged from the only small changes in red cell [Hb] (Fig. 2). This may signify a species difference but could also reflect that the lower metHb levels reached in the present study were below the threshold concentration for activating the changes. The decrease in haematocrit and total blood [Hb] was larger in nitrite-exposed fish than in control fish, probably reflecting an increased removal of disfunctional RBCs in nitrite-exposed fish.

The stoichiometry of the oxidation of oxyhaemoglobin (HbO_2, with haem iron as Fe^{2+}) to methaemoglobin (Hb^+, with haem iron as Fe^{3+}) by nitrite shows that NO_2^- is consumed and NO_3^- is produced in the oxidation process (Kosaka and Tyuma 1987):

$$4HbO_2 + 4NO_2^- + 4H^+ \rightarrow 4Hb^+ + 4NO_3^- + O_2 + 2H_2O$$

The removal of NO_2^- inside the erythrocytes means that the actual uptake of NO_2^- into the blood is larger than indicated by plasma [NO_2^-]. The nitrate ions produced are transported by the RBC anion exchanger (Baroin et al. 1984), contributing to the accumulation of NO_3^- in the plasma (Fig. 3). The faster rise in plasma [NO_3^-] in group 1 than in group 2 mirrors the different NO_2^- accumulation rates. Nitrate may, however, be produced by processes other than the oxidation of Hb. In crayfish, nitrite is endogenously converted to nitrate by a process not related to the respiratory pigment, haemocyanin (Jensen 1996). Similarly, rat hepatocytes can oxidize NO_2^- to NO_3^- (Kim and Lancaster 1993). The potential presence of a similar detoxification mechanism in rainbow trout would contribute to elevate plasma [NO_3^-].

Nitrite accumulation resulted in a larger perturbation of the extracellular electrolyte balance in group 1 than in group 2. Plasma [lactate] rose to high levels in group 1 but not in group 2 (Fig. 3). Since group 2 eventually reached similar metHb levels as group 1, it is not the degree of O_2 shortage per se but rather the rate of its development that is important for the rise in plasma lactate. The fast metHb formation in group 1 may have induced escape attempts thus increasing lactic acid formation in muscle.

The decrease in plasma [Cl^-] was substantially faster and larger in group 1 than in group 2 (Fig. 3). The sum of anions, however, remained practically constant in both groups. In group 1, the rises in plasma [NO_2^-], [NO_3^-], [lactate], and [HPO_4^{2-}] accounted for 18.6 mequiv/L of the 22.2 mequiv/L decrease in plasma [Cl^-] after 24 h. The gap of 3.6 mequiv/L is small, considering the measurement variability of each anion, but may be accounted for by a small rise in plasma [HCO_3^-] and pH (increasing the negative charge on plasma proteins) as seen in carp (Jensen et al. 1987). In group 2, the plasma [Cl^-] decrease of 6.2 mM balanced the increases in plasma NO_2^-, NO_3^-, and lactate (~0.5 mM), which summed to 6 mM. In both groups of nitrite-exposed trout, not only the sum of anions but also the sum of cations as well as osmolality were constant,which is in line with findings in nitrite-exposed carp (Jensen et al. 1987).

Fig. 5. Changes in potassium and water contents of muscle and heart tissues in non-catheterized nitrite-exposed rainbow trout. Values are means ± SEs ($N = 12$ in group 1 and $N = 9$ in group 2). Symbols are as in Fig. 1.

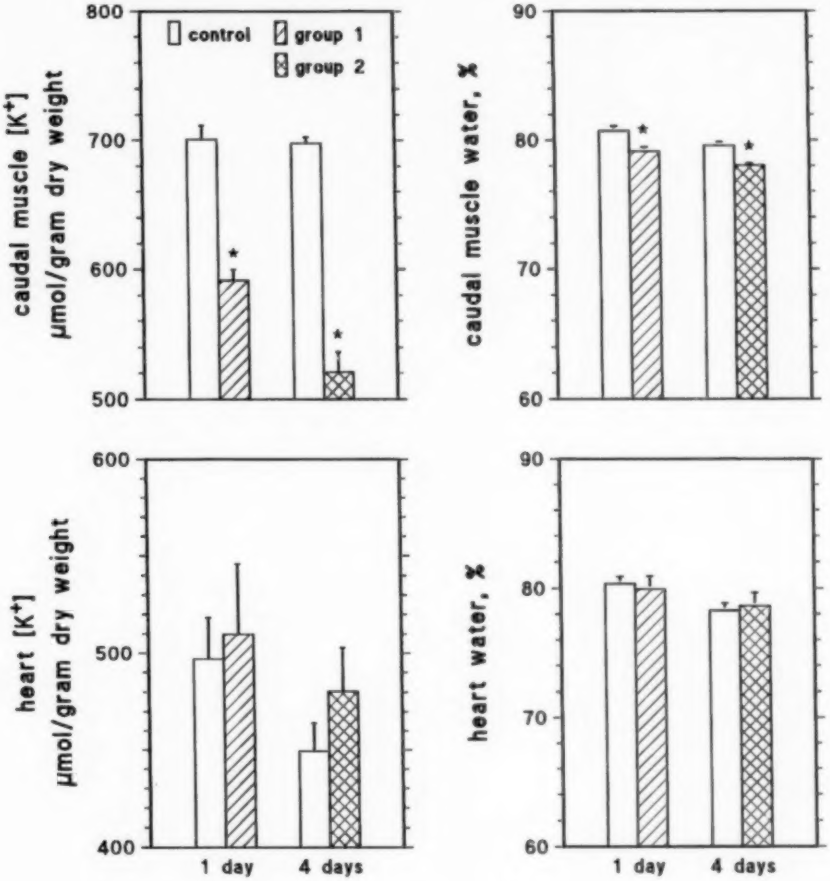
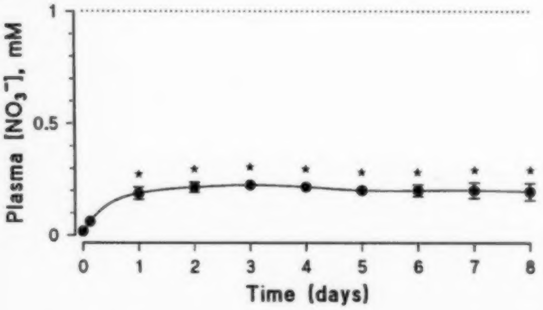


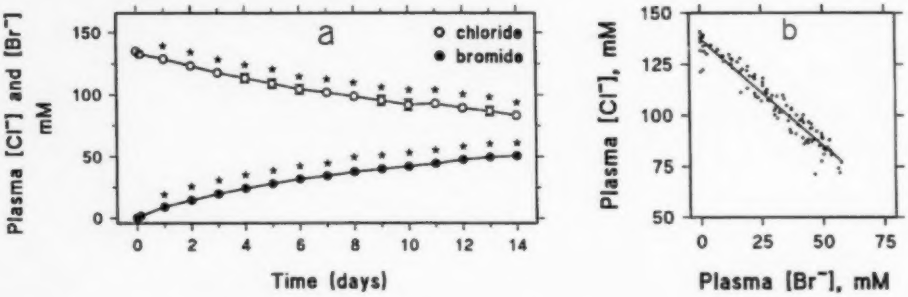
Fig. 6. Plasma nitrate concentration in catheterized rainbow trout exposed to 1 mM ambient NO_3^-. Values are means ± SEs ($N = 5$). Symbols are as in Fig. 1.



The significant increase in plasma [K^+] of group 1 trout (Fig. 4) compares with the extracellular hyperkalaemia induced in carp (Jensen et al. 1987), which was attributed to K^+ efflux from the RBCs and other intracellular compartments (Jensen 1990*a*). Potassium efflux from the RBCs probably contributed little in the present study (cf. above). On the basis of a nitrite-induced decrease in muscle tissue K^+ content of crayfish (Jensen 1990*b*), muscle tissue appeared a likely candidate for K^+ loss also in fish. Indeed, a large significant decrease was observed in the potassium content of caudal muscle in both groups of nitrite-exposed trout (Fig. 5). The muscle K^+ loss was parallelled by a decrease in the muscle water content (Fig. 5), suggesting that the K^+ efflux from muscle resembles that seen from RBCs, by being accompanied by Cl^- and osmotically obligated water.

The loss of K^+ from the muscle tissue was larger in group 2 than in group 1 (Fig. 5), which correlated with a longer NO_2^- exposure time. In contrast with group 1, however, an extracellular hyperkalaemia was not observed in group 2 (Fig. 4). During the extracellular hyperkalaemia following severe exercise, K^+ is lost across the gills in rainbow trout (Wood 1988). Renal excretion also increases, though it is small compared with the branchial loss (Wood 1988). A similar net efflux of K^+ to the surrounding water might occur in NO_2^- exposed trout. The absent extracellular hyperkalaemia in

Fig. 7. (*a*) Time-dependent changes in plasma bromide and chloride concentrations in catheterized rainbow trout exposed to 1 mM ambient Br^-. Values are means ± SEM ($N = 7$). Symbols are as in Fig. 1. (*b*) Plasma $[Cl^-]$ as a function of plasma $[Br^-]$. The regression line has the equation: $[Cl^-] = -1.04[Br^-] + 137.4$ ($r^2 = 0.931$, $P < 0.0001$, $N = 110$).



group 2 could then be a consequence of the slower developing electrolyte disturbance and longer exposure time, allowing the net K^+ efflux from the animal to keep pace with the efflux of K^+ from intracellular to extracellular compartments. This would suggest that the fish experienced a general K^+ depletion during nitrite exposure. Recent data on carp support this idea, since during recovery from nitrite exposure, carp develop an extracellular hypokalaemia (P.K. Knudsen and F.B. Jensen, unpublished data).

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Mesoscale acoustic patterns of juvenile walleye pollock (*Theragra chalcogramma*) in the western Gulf of Alaska[1]

Richard D. Brodeur and Matthew T. Wilson

Abstract: The mesoscale distribution patterns of age–0 walleye pollock (*Theragra chalcogramma*) in the western Gulf of Alaska were assessed through a combined acoustic and midwater trawl survey in 1990. Using a 38-kHz split–beam acoustic system and directed midwater trawling, we were able to isolate concentrations of scatterers that were mainly age–0 pollock based on the expected target strength (–45 to –53 dB) derived from an empirical relationship of target strength versus length. The concentrations of these scatterers formed diffuse layers that were centered at a depth of 33 m during the night. During the day, scatterers descended to a depth of ca. 100 m (up to 30 m off the bottom) where they coalesced into tight aggregations. A sharp thermocline did not inhibit the diel migration of these juveniles. Higher concentrations were found in inner shelf regions as compared with inshore bays or offshore waters, but the varied topography appeared to have little effect on the distribution of these juveniles. These results are discussed in relation to the trophic position of these fish as both predators and prey in this system.

Résumé : Nous avons étudié la répartition à moyenne échelle des jeunes d'âge 0 de goberge de l'Alaska (*Theragra chalcogramma*) du golfe d'Alaska, dans le cadre d'un relevé effectué en 1990 par pêche sélective au chalut pélagique et par détection au moyen d'un système acoustique de 38 kHz à faisceau divisé. Nous avons ainsi pu isoler des concentrations de diffuseurs qui correspondaient principalement à des goberges d'âge 0, étant donné l'intensité cible de –45 à –53 dB que permettait de prévoir une relation empirique entre cette intensité et la longueur des poissons. La nuit, ces concentrations formaient des couches diffuses centrées à une profondeur de 33 m. Le jour, elles descendaient à une centaine de mètres de profondeur (jusqu'à 30 m du fond) et se regroupaient en masses serrées. La présence d'une thermocline abrupte n'inhibait pas la migration journalière des juvéniles. Des concentrations plus élevées de ces poissons ont été relevées dans la partie intérieure du plateau continental que dans les baies intérieures et au large. Cependant, les variations topographiques semblaient avoir peu d'effet sur la répartition des juvéniles. Nous traitons de ces résultats dans le contexte de la position trophique de la goberge de l'Alaska, qui est à la fois proie et prédateur dans le système.
[Traduit par la Rédaction]

Introduction

Walleye pollock (*Theragra chalcogramma*) is the most abundant commercially exploited fish species in the North Pacific Ocean. Juvenile pollock are an important component of the marine ecosystem in the western Gulf of Alaska (GOA) serving as a primary forage item for many marine fishes, mammals, and birds (Hatch and Sanger 1992; Springer 1992; P.A. Livingston, unpublished data; Brodeur and Wilson 1996; Merrick and Calkins 1996). Although larger scale (tens to hundreds of km) distribution patterns of age-0 walleye pollock in the GOA are fairly well known from pre-recruit pollock surveys (Hinckley et al. 1991; Brodeur et al. 1995; Wilson et al. 1996) or from by-catch in surveys targetting other species (Smith et al. 1984; Bailey and Spring 1992), relatively little is known about mesoscale (0.1–10 km) horizontal or vertical aspects of distribution. It is at these smaller spatial scales that many of the interactions between predators and pelagic prey take place (Schneider and Piatt 1986; Schneider 1989).

Little information is available on the diel vertical distribution of age-0 walleye pollock in the GOA. Prior to their primarily demersal existence at age-1 (McKelvey 1996), pollock in the GOA are assumed to be pelagic through at least the first 6 months, but this assumption is based on studies of age-0 pollock in the Bering Sea (Lynde 1984; Bailey 1989; Swartzman et al. 1994). In contrast to the relatively broad flat Bering Sea shelf with its sluggish currents, the juvenile nursery area in the GOA has a more rugged topography, with numerous banks and gullies, and more dynamic and complex oceanographic conditions. Diel and ontogenetic changes in depth distribution have been shown for other populations of age-0 pollock based on acoustic and trawl data (Kamba 1977; Nakatani and Maeda 1987; Bailey 1989), but similar studies have not been conducted for the GOA population. Virtually nothing is known about the aggregation patterns of these juveniles under natural conditions and how these patterns change on a diel basis, although marked day–night differences in group cohesion of age-0 pollock have been observed

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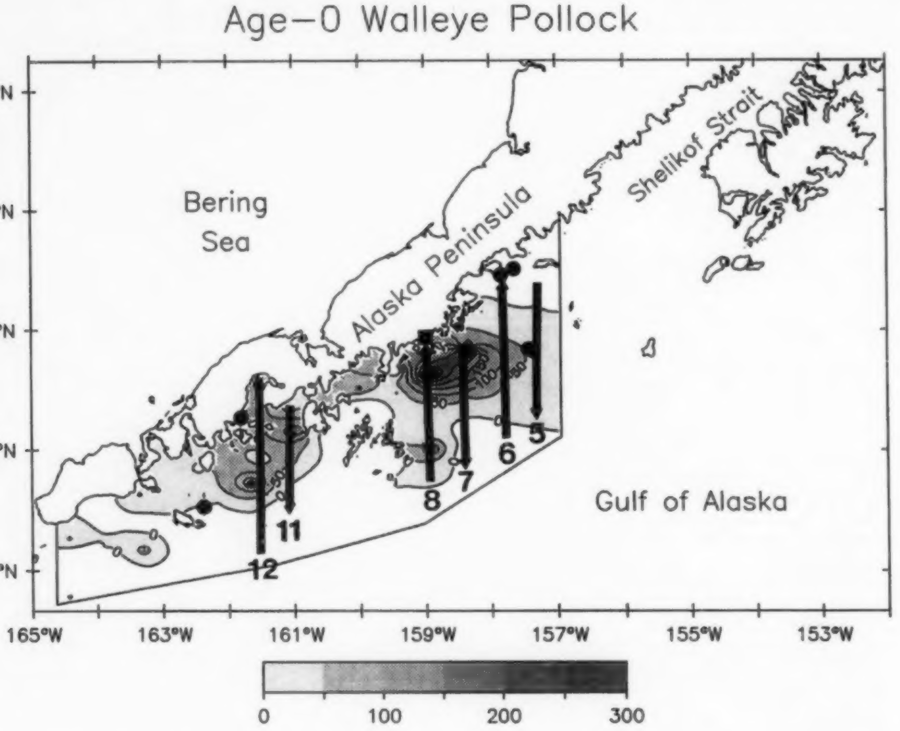
Fig. 1. Map of the study area showing locations of six acoustic transects examined for spatial patterns in age-0 walleye pollock scattering intensity (vertical lines), location of stations examined for target strength information (circles), and the location where the day–night comparison study was undertaken during September 1990 (square). Arrows on transect lines indicate direction of travel during acoustic survey. Also shown are the density isopleths (no. / 100 000 m^3) of age-0 pollock based on a trawling survey conducted at the same time as the acoustic survey.



in the laboratory (Sogard and Olla 1996). In this paper, we analyze quantitative acoustic data from a juvenile pollock survey in the western GOA to examine the inshore–offshore and diel vertical distribution and aggregation patterns of age-0 walleye pollock and compare these patterns with those for other pollock populations.

Materials and methods

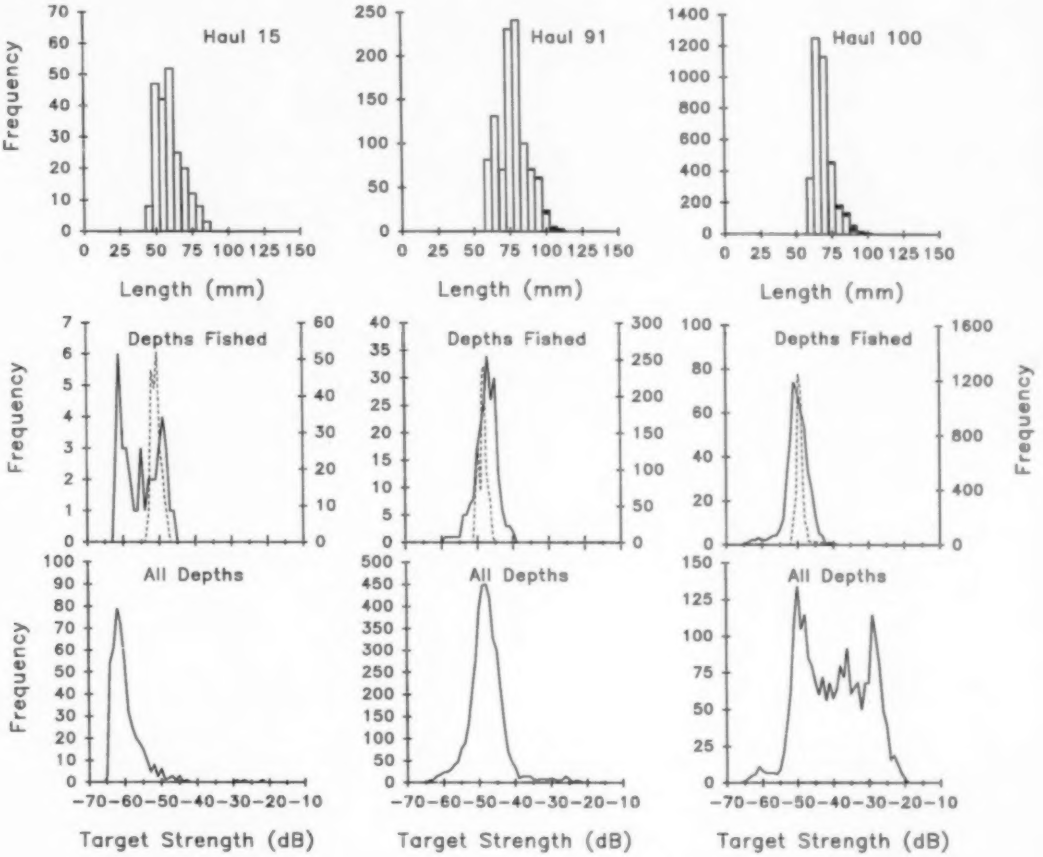
During September of 1990, a combined acoustic and midwater trawl survey was conducted aboard the R/V Miller Freeman to examine the abundance and spatial patterns of age-0 pollock in the western GOA. The results of the trawl survey and a description of the large-scale distribution pattern of age-0 pollock are presented elsewhere (Wilson et al. 1996). Acoustic data were collected along parallel tracklines extending from the Alaska Peninsula to beyond the shelf edge (Fig. 1). The acoustic tracks were occasionally interrupted to trawl either at a predetermined grid station or to identify acoustic sign. After trawling was completed, the trackline was resumed where it had been interupted so that the acoustic transects were spatially, if not temporally, continuous. Ship speed ranged from 18 to 22 km · h^{-1} during transects. Since our main interest was in examining the mesoscale distribution patterns of age-0 pollock, we limited our analysis to six tracklines in areas containing high densities of juvenile pollock (Wilson et al. 1996), although hauls in other areas that caught large numbers of age-0 pollock were used to identify acoustic targets (Fig. 1). Acoustic and trawl sampling was conducted at all times of the day. In addition, one station was sampled repeatedly for a period of 15 h (Fig. 1) to examine day–night differences without the confounding effects of area.

Acoustic data were collected using a quantitative Simrad EK500 echo sounding system. A 38-kHz split-beam transducer was mounted in the centerboard of the ship at a depth of 10 m. The pulse width was set at 1 ms, the nominal beam width was 6.7°, and the acoustic threshold was set at –70 dB. The system was calibrated before and after the survey using a copper ball of known acoustic properties suspended below the ship (Foote et al. 1987). Output data strings from the EK 500 system's echo-integrator were logged as color echograms as well as onto a personal computer for later analysis. Data were logged with a vertical resolution of 1.5 m and a horizontal resolution of ~5 m but were binned into 5-m depth and 3-min time increments for analysis.

The echo-sounding system provides information on both the target strength (TS) of fish and other organisms as well as a measure of backscattering strength. Information on TS was required to infer the identity of scatterers detected in the sections of the transects where no trawls were done. Target strength distributions were examined for the entire water column and also, in the case of trawls that targeted a particular layer, only for those depths through which the mouth of the trawl passed.

Two types of trawls were used, both of which contained small-mesh (3-mm) liners. The primary gear was a high-opening shrimp trawl, towed either in a stepped or oblique manner. Additional oblique collections were made with a pelagic anchovy trawl. The mouth opening of both nets varied according to wire out and ship

Fig. 2. Distribution for individual hauls of length of fish caught (upper panel), target strength (TS) distributions estimated for only the depths fished (middle panel, left axes), and the TS distributions for the entire water column (bottom panel) for the horizontal tows. Also shown are the expected TS distributions (middle panel, broken lines, right axes) based on the observed length distributions and the theoretical length to TS relationship (see text). The open histograms represent age-0 walleye pollock lengths and the filled histograms represent either capelin or eulachon lengths.



speed (nominally set at 6 km · h^{-1}), but the average mouth areas of the shrimp and anchovy trawl were 28 and 140 m^2, respectively (Wilson et al. 1996). The standardized catches of age-0 pollock did not differ significantly between the two gear types, and both nets appeared to be adequate for capturing the full size range (40–110 mm) of fish available (Brodeur and Wilson 1995; Wilson et al. 1996).

To test whether TS was indicative of fish size, we examined the degree of correspondence between TS and size of fish caught. Length frequencies were determined for the entire catch or were expanded from a representative subsample when large numbers of fish were caught. The standardized length distributions of fish caught in relatively monospecific hauls of pollock were converted into an expected TS frequency using an empirically derived relationship for walleye pollock (Foote and Traynor 1988; Traynor 1996):

$$\mathrm{TS}\ (\mathrm{dB}) = 20 \log(L) - 66$$

where L is fork length in cm, and compared with the observed target strength distributions for both the path sampled by the trawl and for the entire water column.

Soule et al. (1995) have shown that the phase algorithm used by the Simrad EK500 does not reliably discriminate single targets in controlled laboratory conditions. They conclude that a bias against weaker targets and erroneous acceptance of multiple echoes would both cause TS values to be higher than expected, especially in high-density areas. We examined this by comparing the trawl densities of age-0 pollock at the stations used in our fish length – TS analysis to the maximum resolution threshold densities (one target per reverberation volume) to avoid multiple target detection. The reverberation volume (V) was calculated as

$$V = c\,\gamma/2\,R^2\,\Psi$$

where c is the speed of sound in water, γ is the pulse length in seconds, R is the depth of targets, and Ψ the solid angle of the transducer's equivalent beam.

In every case, fish density was sufficiently low that only one age-0 pollock per reverberation volume was likely. This, together with the close correspondence between the peak observed and theoretical TS values (Fig. 2), suggests that our TS data were of good quality. We recognize that fish aggregation patterns not resolvable with trawl catch data may have caused small-scale density variations leading to the multiple acceptance bias indicated by Soule et al. (1995). However, our densities were sufficiently low that we feel that this bias was negligible.

Table 1. Definitions of size, density, and location parameters used to characterize patches for statistical comparison.

Parameters	Description
Location	
Depth	Distance in meters from the surface to center of patch
Distance off bottom	Distance in meters from bottom to center of patch
Size and shape	
External height	Maximum number of row elements in a patch
Internal height	Maximum number of consecutive row elements in patch
External width	Maximum number of column elements in a patch
Internal width	Maximum number of consecutive column elements in a patch
Area	Number of elements in a patch
Perimeter	Outside dimension of patch with value of 1 per element side
Fractal dimension	Relationship of perimeter to area
Density	
Mean S_A value	Total S_A of patch divided by area of patch
Peak S_A value	Maximum S_A value in a patch

Note: See Nero and Magnusson (1989) and Barange (1994) for equations used to calculate each parameter.

Mean backscattering area (square metres) per square nautical mile (nm; 1 nautical mile = 1.852 km) of sea surface (S_A) was calculated for each 5-m depth interval as follows:

$$S_A = 4\pi r_0^2 1852^2 \int_{z=r_1}^{z=r_2} S_v \, dz$$

where z is depth, r_0 is the reference range for backscattering strength (1 m), r_1 and r_2 are the top and bottom of the interval, and S_V is the volume backscattering strength (m^2/m^2). The S_A output was first scrutinized for occasions where the bottom detectors failed to reject bottom echoes. These were mainly found in areas where abrupt changes in topography occurred. The edited S_A values were contoured by depth and distance along the transects using a "kriging" anisotropic interpolation algorithm with a linear variogram in SURFER (Windows Version, Golden Software, Inc.). Temperature data, collected at approximately 25-km intervals along the transects using expendable bathythermograph (XBT) casts, were also spatially integrated in SURFER using the same interpolation algorithm, using observed data points collected at 10-m depth intervals.

We were interested in characterizing diel and inshore-offshore differences in the size, shape, and relative density of the aggregations of age-0 pollock determined acoustically. Because of the size of the bins used in calculating S_A values (5 m × ≈1 km), it was not possible to examine fine-scale patchiness, although such patterns were evident on the echograms (see Results); instead, we focussed on the mesoscale patterns evident in the data.

We first needed to set a criterion for determining whether a particular acoustic backscattering intensity and the ones adjacent to it constituted a patch. We first tried setting a variable threshold that would be affected by the surrounding values by fitting an 11-point moving average of the S_A values through the main concentration of targets in each transect attributed specifically to age-0 pollock based on their TS values, similar to that done by Nero and Magnuson (1989). However, since this threshold would show patchiness only at a finer scale than that in which we were interested, we opted for a fixed S_A threshold level for all the transects, during both day and night, so that diel differences in mesoscale aggregation and migration patterns could be more easily assessed. We experimented with several fixed contour levels using SURFER and found that contouring S_A values >50 in increments of 50 provided the most similar patterns to those seen in the original echograms. All contiguous superthreshold cells were included within a "patch."

Following Nero and Magnuson (1989) and Barange (1994), we measured several size and density variables using the original data bins for each patch identified, including external and internal height and width, area, perimeter, fractal dimension, and the mean and peak S_A value (Table 1). We also defined two location variables, depth and distance off bottom, based on the depth in meters that the center of the patch was displaced from the surface or bottom. We calculated means for each variable for both day and night, excluding any patches detected within twilight periods. We also determined means for the inner half (inshore) and outer half (offshore) of each transect.

For all transects combined, differences in patch statistics by time, distance, and their interactions were tested using a two-way ANOVA, with transect as a blocking factor, using a partial confounding design (Heilberger 1989). For some variables, a log or square-root transformation was required to stabilize the variance. For individual transects, *t*-tests were used to detect differences in day–night and inshore–offshore values for each patch statistic.

Results

Target strengths and their identification

Six hauls were selected for examination of TS distributions (Table 2). Three horizontal hauls (15, 91, and 100) targeted a single layer of echosign and were predominantly monospecific for pollock, but several other small pelagic fishes such as capelin (*Mallotus villosus*) and eulachon (*Thaleichthys pacificus*) were also caught. Three oblique to near-bottom hauls (114, 122, and 123) contained large numbers of older age groups of pollock and were examined for contrast.

Fig. 3. Distribution for individual hauls of lengths of fish caught (upper panel) and the TS distributions for the entire water column (bottom panel, solid lines, left axes) for the oblique tows. Also shown are the expected TS distributions (bottom panel, broken lines, right axes) based on the observed length distributions and the theoretical length to TS relationship (see text).

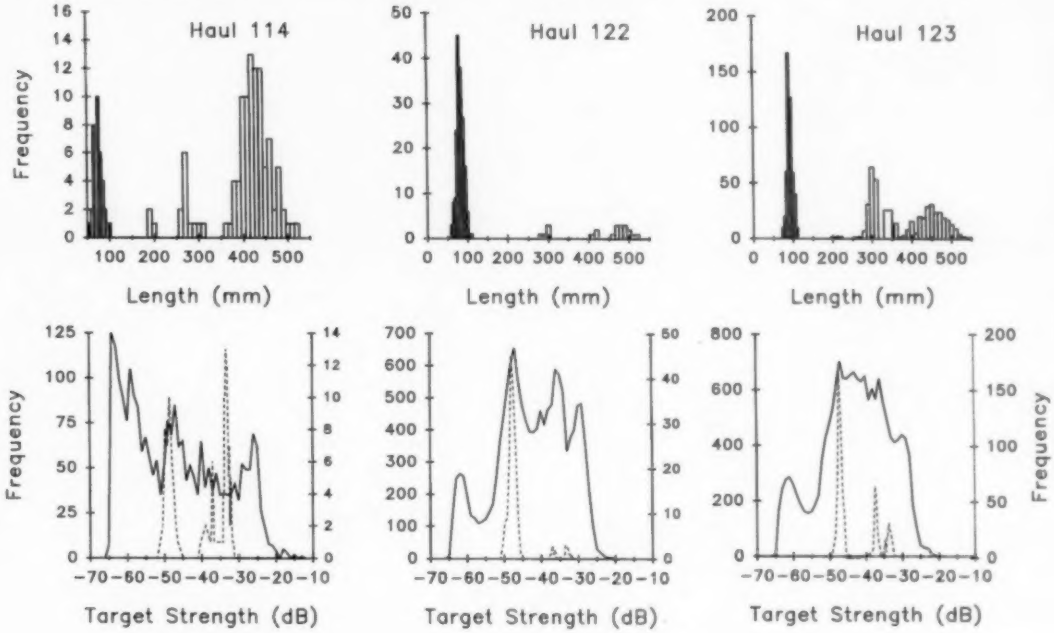


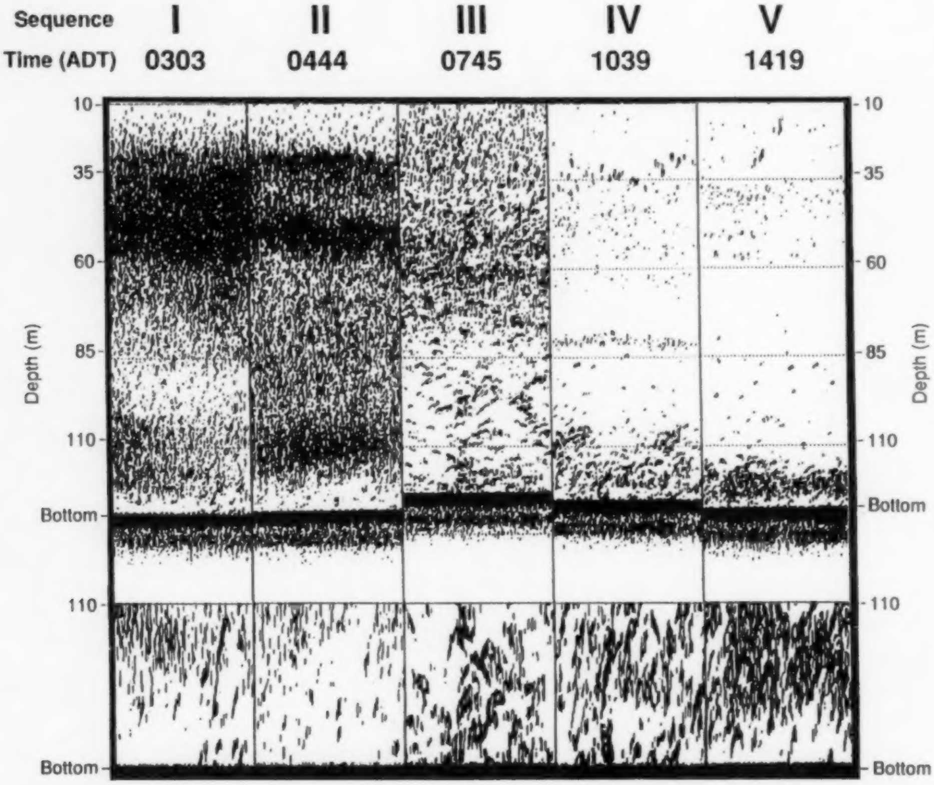
Table 2. Estimated densities (no. / 100 000 m^3) of the dominant fish species and total scyphomedusae collected in hauls examined for echosign interpretation.

	Haul no.					
	15	91	100	114	122	123
Diel period	D	N	N	D	N	N
Type of tow	H	H	H	O	O	O
Maximum depth	88	46	42	102	283	283
Taxon and age group						
Age-0 pollock	701.4	1736.1	6925.1	12.4	176.7	78.2
Age-1 pollock				1.0		1.2
Age-2 pollock				7.2	7.9	31.1
Age-3+ pollock				26.3	16.9	39.6
Adult capelin		25.8	176.4		1.0	
Adult eulachon			31.7	6.1		4.8
Scyphomedusae	54.9	30.1	19.8	23.0	13.6	15.7

Note: The diel period fished (D, day; N, night), the type of haul (H, horizontal; O, oblique), and the maximum depth of tow in metres are given for each station. All other taxa occurred at densities <1.0 / 100 000 m^3.

All three hauls that caught predominantly age-0 pollock displayed a peak at or near –50 dB in TS distributions for the path of the tow. These peaks were within 3 dB of those predicted for juvenile pollock of this size based on the empirical length–TS relationship (Fig. 2). Haul 15, which caught age-0 pollock and a moderate amount of medusae, also showed another TS peak at approximately –62 dB for both the depths fished and for the total water column. The three tows that caught several age-groups of pollock were fished obliquely throughout the entire water column (Table 2); thus, it was difficult to examine target strengths just for the path of the tow. Although the acoustic data collected during these tows showed a broad range of TS distributions, all three tows contained a peak within a few decibels of the expected juvenile peak (Fig. 3). The other peaks were appropriate TS values (20 Log L – 66) for older age groups.

Fig. 4. Sequence of 38-kHz echograms taken at different times of the day but at the same station. The top part shows the entire water column and the bottom shows an expansion of the bottom 10 m of the water column. Each sequence corresponds to a 3-min time interval and a distance of approximately 1 km.



Diel patterns of distribution

The acoustic patterns observed at the station sampled at different times of the day showed substantial differences in fish distribution and aggregation patterns between night and day observations (Fig. 4). The first two panels show a typical nighttime pattern with one or two pronounced layers in midwater (30–60 m) and a diffuse layer 10–20 m off bottom. Shortly before dawn (which occurred at 08:10 ADT), the surface layers had begun to move down (middle panel of Fig. 4) and by day, the primary layer was concentrated in the lower 10–20 m of the water column with only diffuse layers elsewhere (last panel of Fig. 4).

The layers remaining in the water column during the day consisted of scatterers of relatively low TS (–64 to –57 dB), which we interpreted to be mainly large scyphomedusae based on the trawl catches. Examination of the backscattering intensity by 5-m depth intervals at different times indicated that, by day, almost all the biomass was near the bottom, whereas during nighttime the majority of the biomass was in midwater (left panel of Fig. 5).

We next examined the vertical distribution of just the individual scatterers that registered target strengths between –53 and –45 dB, estimated to correspond roughly to age-0 pollock from 45 to 110 mm in length. The depth at which the peak abundance of this size fish was detected increased consistently during this period, progressing from 25 m in the middle of the night to 110 m during the day (middle panel of Fig. 5). Although there was not a sharp thermocline apparent at this location (right panel of Fig. 5), this layer experienced in excess of a 3°C change in ambient temperature during this diurnal movement. Based on TS measurements (TS ≈ –35 dB), the layer that remained in deep water during the night (Figs. 4 and 5) consisted mainly of older age groups of pollock (≈48 cm; Traynor 1996).

Numerically, age-0 walleye pollock (53–107 mm) dominated the finfish catch (>74% of total) and were found in all of the collections (Table 3). Eulachon was the only other abundant small pelagic species, but the size range (114–196 mm) of individuals caught did not overlap that of age-0 pollock. In terms of biomass, however, older age groups of walleye pollock and large scyphomedusae comprised the bulk of the catch in these trawls.

Patterns in the distribution of scatterers from individual transects showed much variability depending on time of day. We present the distributions for the three transects that contained the highest densities of age-0 pollock, although the other three transects show the same general patterns.

Transect 7 (17:22–02:10 ADT) clearly illustrated a transition from day to night (Fig. 6). The layer (predominant TS ≈ –50 dB) generally left the bottom around sunset and migrated

Fig. 5. Vertical distribution in 5-m bins of overall backscattering intensity (left panel) and the number of acoustic targets between TS values of –45 and –53 dB (center panel) from the same location at different times of the day. Open symbols represent daytime and solid symbols represent nighttime observations. Also shown are the corresponding day–night temperature profiles at this location (right panel).

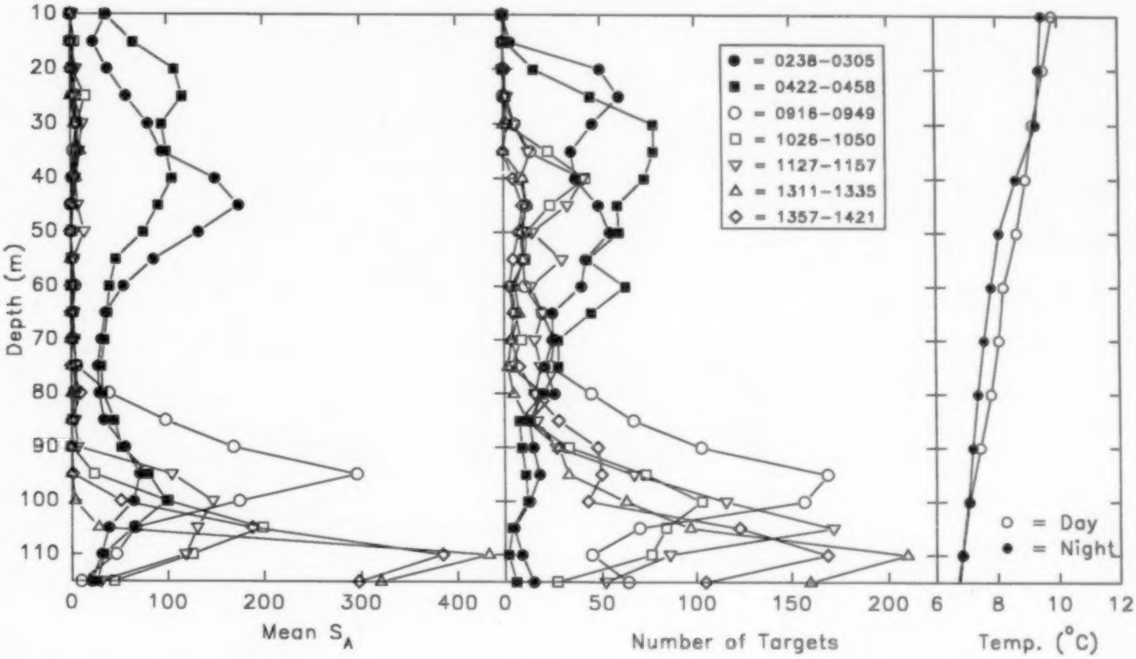


Table 3. Number of occurrences, mean densities, and mean weights of the dominant fish and invertebrates from six hauls at the same location during September 1990.

Species	Positive hauls	Mean no./ 100 000 m^3	Mean weight/ 100 000 m^3 (kg)
Age-0 walleye pollock	6	33.15	0.12
Age-3+ walleye pollock	4	7.44	4.69
Capelin	1	1.69	0.25
Eulachon	3	2.58	0.09
Scyphomedusae	6	7.05	1.28

Note: All other taxa caught occurred at densities lower than 1.0 per 100 000 m^3.

to the surface in about 2 h. Some scatterers remained near the bottom during the night, but these aggregations appeared to be mainly adult fish based on their TSs (–38 to –29 dB). A sequence of echograms produced during this transect (Fig. 7) showed both a change in the vertical position of this layer and the aggregation pattern that was typical of many of the echograms. During the daytime, the layer occurred over a narrow depth range (5–10 m) and was made up of discrete aggregations (sequence I). This layer gradually diffused as it rose in the water column and spread over a 25-m depth range, and the aggregations disappeared (sequences II–IV). It is also apparent that some of the layer may have been above the transducer face (10 m) during nighttime (e.g., Figs. 6 and 7).

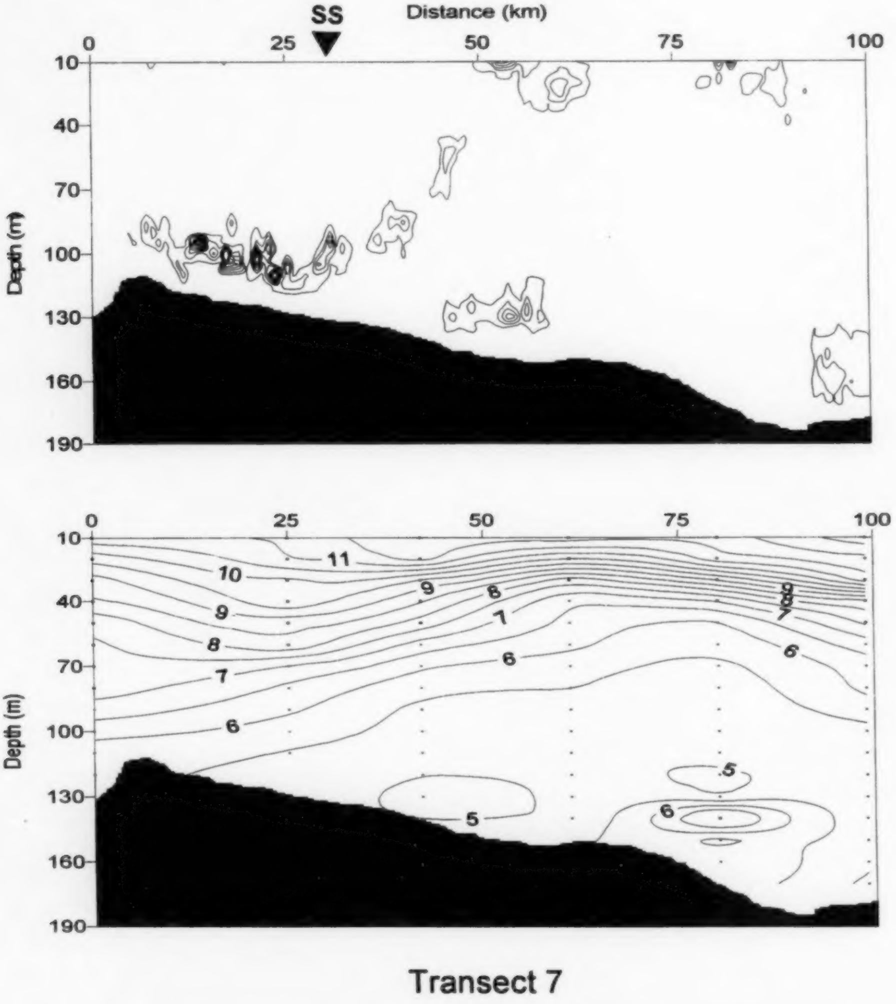
Transect 8 (04:40–17:27 ADT) showed the downward migration of the layer, which began at sunrise and continued for about 2–3 h (Fig. 8). The layer remained in close proximity to the bottom despite the varied topography present during this transect. Transect 12 (14:06–03:49 ADT), which was the only transect to extend well inshore and almost to the shelf break (Fig. 1), again showed the diurnal concentrated aggregations near bottom and the nocturnal rise to the surface (Fig. 6). Few scatterers were observed either inshore or offshore on this transect.

Statistical analysis of aggregations

None of the 10 variables examined showed significant interactions between time and distance-offshore effects, which allowed us to examine these effects independently. For the five transects that had both day and night observations, only the depth and distance off bottom of the patches showed significant differences for all transects combined (both $P < 0.001$) with all transect daytime patch depths greater and closer to the bottom than at night (Table 4). When we combined all day patches and all night patches together and then weighed the importance of each patch by its total backscatter intensity, we found the daytime mean depth of patches (99.2 ± 40.0 m; mean ± SD) was significantly ($P < 0.0001$) greater than the nighttime mean depth (33.1 ± 17.5 m). Similarly, the mean distance of patches off bottom during the day (28.9 ± 20.3 m) was significantly ($P = 0.0001$) less than the night mean distance off bottom (65 ± 32.9 m).

The size and shape variables almost consistently showed that patches had larger dimensions at night but, only in a few

Fig. 6. Inshore–offshore pattern of S_A for transect 7 (top panel). The contour levels of S_A start at 50 m^2/m^2 and are incremented by 50 with each successive contour. Time of sunset (SS) is indicated. Also shown is the contoured temperature section (bottom panel) with depth and distance along the transect. The temperature data used in the contouring were taken at the depths indicated by dots in the lower panel.



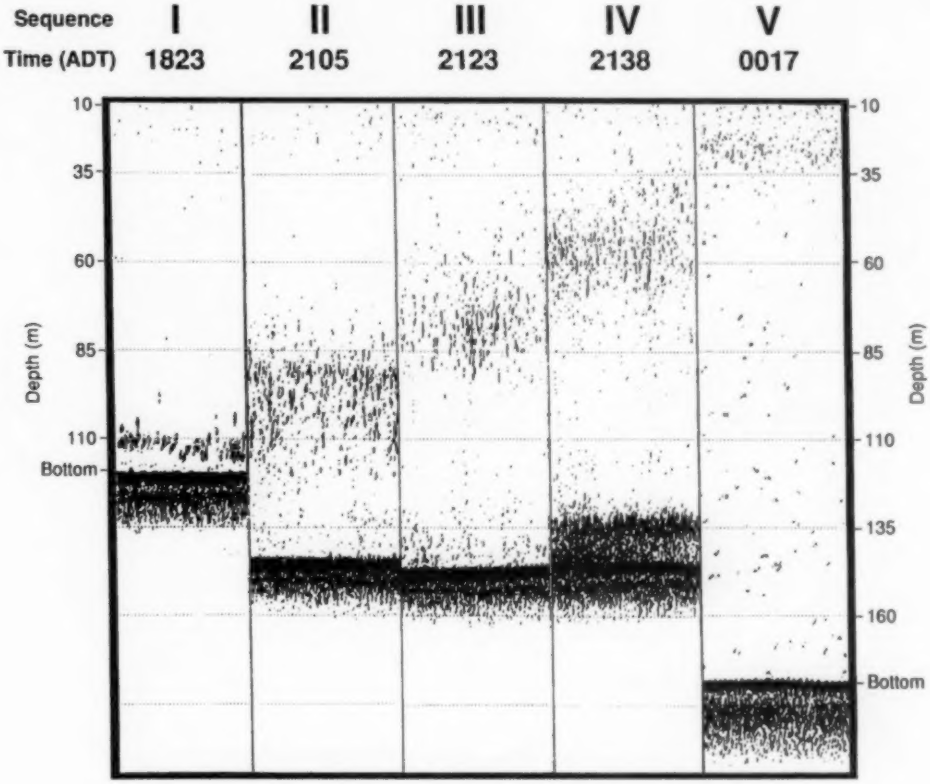
individual cases, were these significant and none of these variables was significant over all transects. All but one of the mean S_A values were higher during the day, perhaps reflecting a partial migration of the layer above the transducer beam during the night. The maximum S_A values, however, showed no consistent trends between day and night (Table 4).

For the inshore–offshore comparisons, the patterns were not consistent for any of the variables. Both the mean depth and mean S_A of the patches tended to be greater inshore than offshore, but there were exceptions.

Discussion

Hydroacoustic surveys are routinely used to estimate the abundance of older age groups of walleye pollock as well as other commercially important species in Alaskan waters (Traynor 1987; Karp and Traynor 1989; Karp and Walters 1994; McKelvey 1996). Up to the present, acoustics have not been used to assess age-0 pollock <100 mm because of uncertainties in the TS range, as well as the schooling characteristics of this age-group and other similar-sized acoustic targets. There is some question as to the suitability of using the TS–length relationship as the resonant object approaches the acoustic wavelength (±3.9 cm at 38 kHz) as is likely for juvenile fishes (M. Barange, personal communication). Although no experimental measurements of age-0 pollock TS have been made, our findings of a peak in TS distribution of around −50 dB in tows that caught almost exclusively age-0 pollock support the extrapolation of the adult TS–length relationship to juvenile pollock (Traynor 1996). Similar in situ

Fig. 7. Sequence of 38-kHz echograms taken at different times of the day along transect 7. Each sequence corresponds to a 3-min time interval and a distance of approximately 1 km.



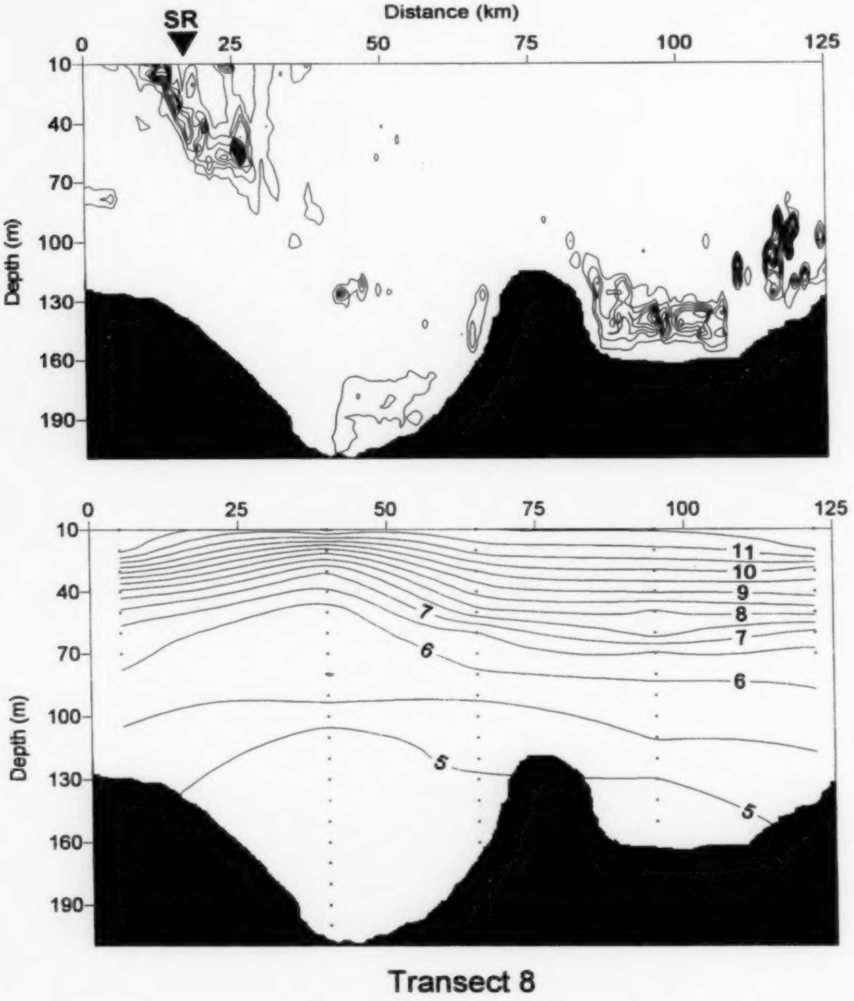
TS values were determined for age-0 Atlantic cod (*Gadus morhua*; mean TS of –55.1 and –57.1 dB for 50-mm fish) in Norway using the same split-beam echo sounder that we used (Ona 1994). Our TS values are slightly higher but still comparable with those found by Barange et al. (1994) for juvenile Cape hake (*Merluccius capensis*). The peak values that we observed were within ±3 dB of the theoretical expectation, but such differences could easily be accounted for by diel differences in tilt-angle orientation (Traynor and Williamson 1983), physiological variations due to condition or stomach fullness (Ona 1990), behavioral changes due to ship or trawl passage (Olsen 1990; Barange and Hampton 1994), or to biases on the size of fish caught due to trawl selectivity (Engås and Soldal 1992; Karp and Walters 1994).

In situ TS measurements are derived ideally in conditions where fish are relatively dispersed and where monospecific schools of relatively uniform size are available. For age-0 walleye pollock, the best data would come from night observations when the pollock are in low densities in surface waters close to the transducer, where biases resulting from the acoustic noise threshold effect and multiple target detection are less likely to be important (Traynor 1996; Soule et al. 1995). Because TS values were used to select patches, some high-density daytime aggregations near bottom may have been affected by the the multiple-target bias (Soule et al. 1995) and may have been omitted from the patch analysis. If any relationship exists between fish density during the daytime and patch volume, shape, or vertical distribution, then our estimates of daytime patch characteristics may have been influenced by these omissions. We did not observe anything to suggest these relationships exist. Thus, the identification of patches as age-0 pollock appears to be reliable enough to use to examine distribution and aggregation patterns.

Although we used acoustic data to describe the distribution and behavior of age-0 pollock, one could derive biomass estimates in some areas where contamination by other small pelagic species, such as eulachon or capelin, is minimal. Preferably, biomass should also be estimated at night when schools are off bottom and not tightly aggregated to minimize acoustic shadowing that occurs with large aggregations (Appenzeller and Leggett 1992; Barange et al. 1994). However, we recognize that the variance associated with such an estimate could be quite large since a 3dB difference in TS may translate into a 100% change in estimated biomass. Ideally, some combination of acoustics and midwater trawling would probably provide the best estimates of abundance for juvenile pollock (Godø and Wespestad 1993; Karp and Walters 1994).

Based upon our trawl and acoustic data, a general descrip-

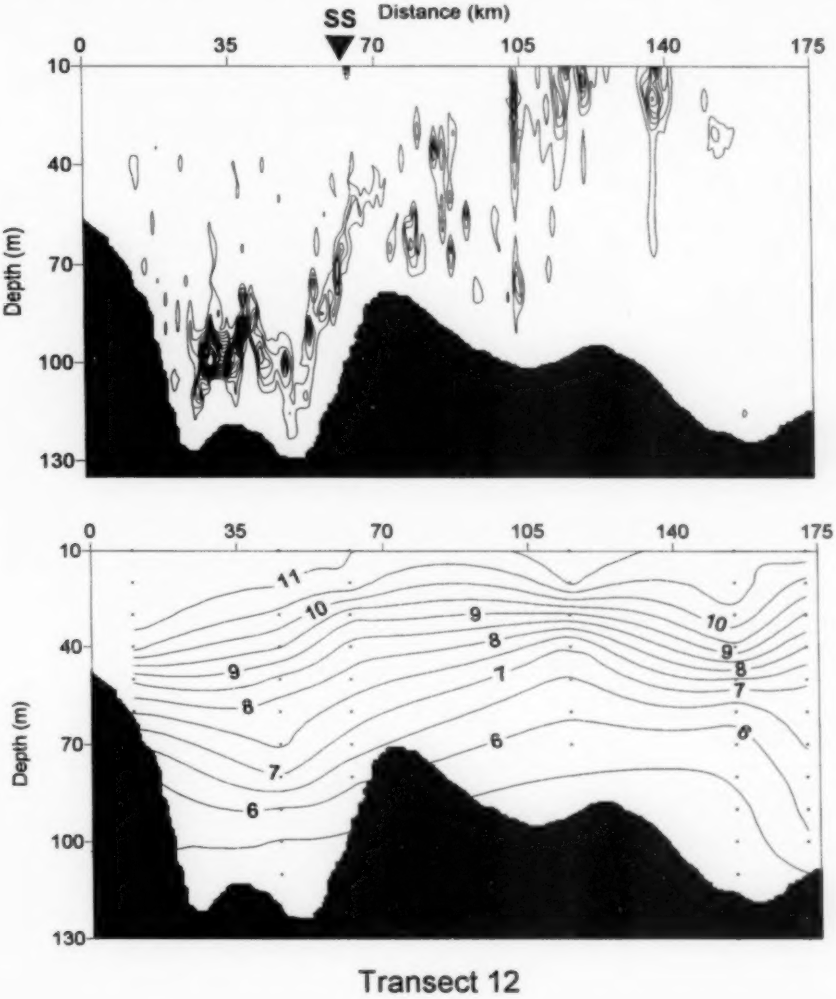
Fig. 8. Inshore–offshore pattern of S_A and temperature for transect 8. See Fig. 6 for explanation of contours. Time of sunrise (SR) is indicated.



tion of the diel distribution pattern of age-0 walleye pollock (50–100 mm) in the GOA emerges. Most age-0 pollock at this size are not associated with the bottom substrate, as is typical of many other gadoid fishes (Lough et al. 1989; Lough and Potter 1993). During daylight hours, juvenile pollock are found in deep water, yet still within the pelagic zone. They tend to form either discrete ball-like aggregations 5–10 m in diameter or else form a dense layer around 10 m thick beginning approximately 5 m above bottom. Just prior to local sunset, they begin to move toward the surface arriving at the 10- to 40-m depth strata within about 1 h. During the night, juvenile aggregations tended to disperse and form one or two layers extending 20–30 m in the vertical. Shortly before dawn, the layers left the surface and again coalesced into dense aggregations above the bottom during daylight hours. This pattern of behavior appears to be distinctly different from that of age-0 pollock collected in the winter months (10–12 months old), which remain close to the bottom both day and night (McKelvey 1996; Brodeur and Wilson 1996).

Although a number of hypotheses have been put forth to explain some adaptive benefit of diel vertical migration in marine fishes that would exceed the energetic costs involved, recent studies have suggested multiple causality in that there may be trade-offs among several factors such as feeding, temperature optimization, and predator avoidance (Bailey 1989; Neilson and Perry 1990; Olla and Davis 1990; Sogard and Olla 1993). Juvenile pollock collected during this cruise fed at night in surface waters (Merati and Brodeur 1996) and the nighttime aggregations tend to be relatively loose, possibly because individual fish may be pursuing prey that are not aggregated. Tilt angles would also be expected to be more variable during the feeding period, reflecting the variety of bearings juveniles use to pursue prey. This is in agreement with the more dispersed distributions seen in the echograms

Fig. 9. Inshore–offshore pattern of S_A and temperature for transect 12. See Fig. 6 for explanation of contours. Time of sunset (SS) is indicated.



and wider range of target strengths observed during the night. However, since the fish are deeper during the day, some of the smaller individuals may not be detected due to the threshold effect, which would result in truncated TS distributions.

During the day, juvenile pollock possibly avoid predation from surface-feeding marine birds and mammals (Hatch and Sanger 1992; Merrick and Calkins 1996) by retreating to the deeper layers. However, adoption of this migration strategy exposes juveniles to many bottom-dwelling fish predators (P.A. Livingston, unpublished data) that do not undertake extensive diel vertical migrations and that may capitalize on these high juvenile pollock densities near bottom during the daylight hours (Bailey 1989). A movement of some large scatterers off the bottom during the daytime is evident in many of the echograms. These scatterers are most likely larger pollock, which were prevalent in bottom trawl collections during this survey (Wilson et al. 1996). Some cannibalism by adult pollock and predation by other fishes has been detected from predators collected in these bottom trawls (Brodeur and Bailey 1996), but the data are insufficient to conclude that these juveniles were exposed to a substantial predation risk near the bottom during this time of the year. However, P.A. Livingston (unpublished data) documented substantial predation by demersal predators, such as adult pollock and arrowtooth flounder (*Atheresthes stomias*), during the summer months prior to our survey.

The presence of a strong thermocline on many of these transects did not appear to affect the vertical migration of age-0 pollock. In all cases, the pollock migrated through the thermocline at the appropriate time, experiencing up to a 6°C change in thermal habitat. Bailey (1989) proposed that the interaction between food availability, temperature and energetics, may determine the vertical migration patterns of juvenile pollock in the Bering Sea, and suggested that, in food-limited areas, pollock are more likely to pass through a thermocline in search of food. A similar response was observed in

Table 4. Mean value of each variable examined for day–night differences in location, size, and acoustic intensity of aggregations found for each transect during 1990.

	Transect 5		Transect 6		Transect 7		Transect 8		Transect 11		Transect 12	
Variable	Day (15)	Night (21)	Day (27)	Night (36)	Day (7)	Night (11)	Day (16)	Night (6)	Day (0)	Night (32)	Day (41)	Night (16)
Depth	74.8*	40.5	118.8*	40.8	65.9*	26.3	121.6*	64.8	—	24.7	64.6*	46.1
Distance off bottom	42.3	49.6	63.8	69.3	51.9	146.4*	35.6	54.3	—	69.7	27.3	56.1*
External height	1.4	2.3*	2.2	3.4	2.7	3.6	2.5	5.2	—	3.4	2.1	3.6*
Internal height	1.3	2.3*	2.2	3.0	2.2	3.4	2.3	3.8	—	3.4	1.9	3.6*
External width	1.1	2.7	2.2	2.2	5.0	2.2	2.9	7.2	—	4.1	1.9	3.3
Internal width	1.1	2.4	2.2	2.0	4.0	2.0	2.7	5.5	—	4.0	1.8	3.3*
Area	1.5	6.3	5.2	7.5	11.6	8.7	8.0	31.5	—	20.5	4.1	8.9*
Perimeter	5.1	11.4	9.4	11.6	19.3	22.7	11.4	27.7	—	17.5	8.3	15.1*
Fractal dimension	0.36	0.72	0.52	0.82	0.56	0.74	0.71	1.04	—	0.53	0.50	1.08*
Mean S_A value	128.7	89.3	123.7*	86.1	121.7	134.2	139.1	130.8	—	81.9	136.8	144.5
Peak S_A value	149.9	195.0	254.3*	114.1	248.0	185.6	225.7	549.5*	—	151.0	265.2	275.7

Note: Asterisks show those values that are significantly (*t*-test, $P < 0.05$) larger than the corresponding value in the opposite time period. Values in parentheses are the number of patches observed in each time period. All of transect 11 was sampled during night.

the laboratory where juvenile pollock generally remained above the thermocline but were motivated to go below the thermocline when food was introduced there (Olla and Davis 1990) or a predator was introduced from above (Sogard and Olla 1993). Little is known, however, about the vertical distribution of prey resources in the GOA during the fall months.

Although, in almost all cases, the mean value of the patch size variables (height, width, area, etc.) was greater during the night than in daytime, few of these were significant because of the substantial variability in school size encountered. The horizontal resolution (≈1 km) of our data bins was probably too coarse to pick up the smaller scale (ten of meters) aggregation patterns observed in the echograms (e.g., Fig. 4). However, substantial day–night differences in aggregation patterns were evident with the daytime signal appearing as concentrated schools and the nighttime pattern appearing as diffuse layers. A similar diel pattern of group cohesion in age-0 walleye pollock was observed in laboratory conditions (Sogard and Olla 1996). Since feeding by these age-0 individuals occurred at night, it is plausible that pollock juveniles disperse to feed in midwater at night and aggregate tightly at depth during the day when they are not feeding. The latter behavior may serve as an antipredation mechanism whereby the formation of tight aggregations minimizes the risk of detection and capture by visual predators (Pitcher and Parrish 1993).

Acknowledgments

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Mechanisms contributing to variable growth in juvenile southern flounder (*Paralichthys lethostigma*)

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Abstract: We evaluated mechanisms accounting for skewed and potentially bimodal sizes among juvenile (age-0) southern flounder, *Paralichthys lethostigma*, in Pamlico Sound, North Carolina. The birth-date distribution of age-0 southern flounder in 1990 was unimodal with a peak in mid-January and birth date and size were unrelated in the juvenile stage. Differential growth, rather than birth date, accounted for the broad dispersion of lengths occurring in the 1st year. By September 1990, females on average reached larger sizes, but they overlapped the whole size range of males; there was evidence for a bimodal juvenile distribution with females dominating the upper size mode. Based on otolith increments, growth rate variation among juveniles through the summer was high, ranging from 0.35 to 1.5 mm per day with an apparent bifurcation in the growth curve beginning at about 75–100 mm total length which corresponded to the onset of piscivory. While variation in the onset of piscivory was the most likely mechanism generating skewed flounder sizes, differential growth may have been magnified because of mismatch of prey size and availability, or environmental factors affecting spatial segregation of small and large individuals from the age-0 cohort.

Résumé : Nous avons évalué les mécanismes pouvant expliquer la distribution désaxée et peut-être bimodale de la taille des juvéniles (âge 0) de cardeau du sud, *Paralichthys lethostigma*, du Pamlico Sound, en Caroline du Nord. En 1990, les dates de naissance de ces juvéniles présentaient une distribution unimodale culminant à une croissance différentielle. Dès septembre 1990, les femelles atteignaient en moyenne une taille plus élevée, mais la plage des tailles recoupait celle observée pour les mâles. Les tailles semblaient présenter une distribution bimodale dont le mode supérieur était dominé par les femelles. Selon l'examen des cernes d'accroissement des otolithes, le taux de croissance variait beaucoup parmi les juvéniles pendant tout l'été, allant de 0,35 à 1,5 mm par jour; la courbe de croissance présentait une bifurcation apparente à partir d'environ 75 à 100 mm de longueur totale, stade correspondant au début du piscivorisme. La variation observée quant au début de ce mode d'alimentation est la cause la probable de la distribution désaxée des tailles, mais la croissance différentielle a pu être amplifiée par le non-appariement de la taille et de la disponibilité des proies ainsi que par des facteurs environnementaux influant sur la ségrégation spatiale des sujets grands et petits d'âge 0.
[Traduit par la Rédaction]

Introduction

The distribution of sizes of individuals in a population has important implications for its dynamics. Many factors such as mortality, growth potential, and migration behaviors depend on body size (Quinn and Brodeur 1991; Crowder et al. 1992). Size structure can dictate the outcome of predator–prey relationships that may be critical in early life history (Persson 1988; Wilbur 1988; Crowder et al. 1992). An apparent example of this is the need in fish stocking programs for matching predators with the "right-sized" prey to maintain a balanced system (Ney and Orth 1986). Often, mechanisms that contribute to a skewed or multimodal size distribution are not well understood, however, and the interaction of several mechanisms may affect the size patterns observed in natural populations (Huston and DeAngelis 1987; Lomnicki 1988).

Although there is little information to support a hypothesis of genetically distinct subpopulations of southern flounder, *Paralichthys lethostigma*, their growth rates may vary regionally, and conflicting evidence on growth and size suggests wide variability in individual growth rates early in their life history (Gilbert 1986). For example, the analysis of size at first otolith annulus by DeVries (1981) showed that young-of-the year (age-0) flounders developed a bimodal size distribution in North Carolina. This apparent bimodality was tentatively attributed to a delayed or "lost" annulus in the larger mode (DeVries 1981), but subsequent work has not supported this hypothesis (Wenner et al. 1990). Age-0 southern flounder reached a mean length of 130 mm by December in North

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Carolina (Powell and Schwartz 1977). In South Carolina, age-0 southern flounder lengths were bimodal with peaks of length distributions at 50 and 140 mm in June (Wenner et al. 1990). In Texas, Stokes (1977) found sexual dimorphism in growth rates: males reached 230 mm and females 300 mm by the first annulus. The perception that growth in excess of 1 $mm \cdot d^{-1}$ was unlikely in Texas waters led to the deletion of all tag-recovered flounders exhibiting growth rates greater than 1 $mm \cdot d^{-1}$ from an analysis of yield (Matlock 1991). But juvenile southern flounder can grow rapidly (1.3–1.4 $mm \cdot d^{-1}$) when fed to excess in rearing trials (Reichert and Van der Veer 1991; Fitzhugh and Rice 1995).

Several plausible mechanisms could contribute to variation in body size and account for the development of a skewed or bimodal distribution in a cohort of fish. A bimodal size distribution could reflect a broad or bimodal birth-date (BD) distribution. If growth is highly variable among individuals or locations, differential growth could account for the development of bimodal size distribution for young fish. Potential factors contributing to differential growth among individuals include sexually dimorphic growth, ontogenetic changes (e.g., onset of piscivory), or habitat-related differences, such as food availability or oxygen levels (Huston and DeAngelis 1987).

We examined the formation of size structure and potential mechanisms underlying growth rate variability among individuals of southern flounder. We focused on the 1990 year-class as we could follow this cohort through the juvenile and adult stages because of an overlap of fishery-independent and -dependent surveys conducted by the state of North Carolina. We aged flounders from these surveys to identify individuals of the 1990 cohort and establish size-at-age and growth differences among sexes. To focus on possible events affecting growth rate variability among individuals during their first summer, we examined daily growth rates and the relationship of BD to body size using otoliths. Finally, we surveyed diet to define the onset of piscivory.

Study area and methods

Our study area, Pamlico Sound, Core Sound, and adjoining tributaries including the Neuse and Pamlico rivers, North Carolina, U.S.A. (approximately 34°30′–36°0′N, 75°30′–77°0′W) has a maximum depth of about 7.3 m with a mean depth of 4.9 m (Giese et al. 1979). Lunar tides are negligible, and water levels are primarily affected by winds (Giese et al. 1979). The system is correspondingly low in salinity, well mixed, and does not stratify except in the lower reaches of the rivers during summer (Stanley and Nixon 1992). Surface salinities, typically 6–10‰ at the mouths of the Neuse and Pamlico rivers, range to 20–25‰ at Ocracoke Inlet (Giese et al. 1979).

We collected juvenile southern flounders from the 1990 year-class by sampling trawl catches during surveys conducted by the State of North Carolina Division of Marine Fisheries (NCDMF). These surveys were designed for multiple purposes, but a major objective was to monitor annual recruitment of important commercial species including southern flounder. The nursery survey has been described by DeVries (1985) and West and Wilson (1994) and uses two trawl sizes: (1) a 3.2-m headrope otter trawl (6.4-mm bar mesh wings and body and 3.2-mm bar mesh cod end) used in upper estuarine reaches to a depth of about 0.6–1.2 m and (2) a 6.1-m headrope otter trawl (19-mm bar mesh body with 6.4 mm bar mesh cod end) used in open embayments and mouths of tributary creeks to a maximum depth of approximately 5 m. The small trawl was pulled for 1 min at 1.1 m/s covering an approximate distance of 69 m while the 6.1 m headrope trawl was towed 5 min at 1.1 m/s for a distance of approximately 343 m. We sampled monthly with NCDMF nursery survey program during the spring, summer, and fall of 1990 and collected flounders from 104 stations (generally at depths less than 1.8 m).

Juveniles from the 1990 year-class were also sampled during the NCDMF survey of sounds during 2–3 weeks in June and September. Stations were chosen based on a random stratified sampling plan (six to eight strata, identified by depth and geographic location; West and Wilson 1994). Twenty-minute tows were taken with double rigged demersal, mongoose trawls (9.1-m headrope, 2.2-cm bar mesh body, 1.9-cm bar mesh cod end; these are tandem trawls commonly used for commercial shrimping). We collected southern flounder from 103 sites during March, June, and September of 1990.

Fishery-dependent samples of flounders from the 1990 year-class were collected during years 1991–1993. These samples were taken from several seasonal fisheries that use gears including shrimp trawls and long-haul seines (summer), gill nets (summer and fall), pound nets and spear fishing (fall), and crab trawls (fall and winter). Flounders were collected during each month fish were available from each of the following size categories; 0–250, 251–350, 351–450, 451–550, and >551 mm total length (TL), and sex was determined only on those fish purchased by the study.

During sound and nursery collections, we placed southern flounder into an ice slurry while in the field, subsequently froze them, and later thawed specimens for examination and measurements. Total lengths were measured to the nearest millimeter and total weights were recorded to the nearest 0.01 g. Fresh lengths and weights (to the nearest gram) were recorded during fishery-dependent sampling. We scored sex as male, female, or undifferentiated after we macroscopically examined each gonad. Whole sagittae were extracted and examined for annuli under magnification by immersing the otolith concave side up in 95% ethanol in a black Petri dish and illuminating with reflected light. We scored annuli according to the guidelines developed by Powell (1982) and Wenner et al. (1990). For the nursery and sound surveys, some flounders were captured in the spring before annulus formation was completed, and we differentiated age-0 flounders from yearlings not showing a completed annulus by observing the difference in length frequency that was evident between these two cohorts (e.g., at least twofold differences in size during March, April, and May; Wenner et al. 1990).

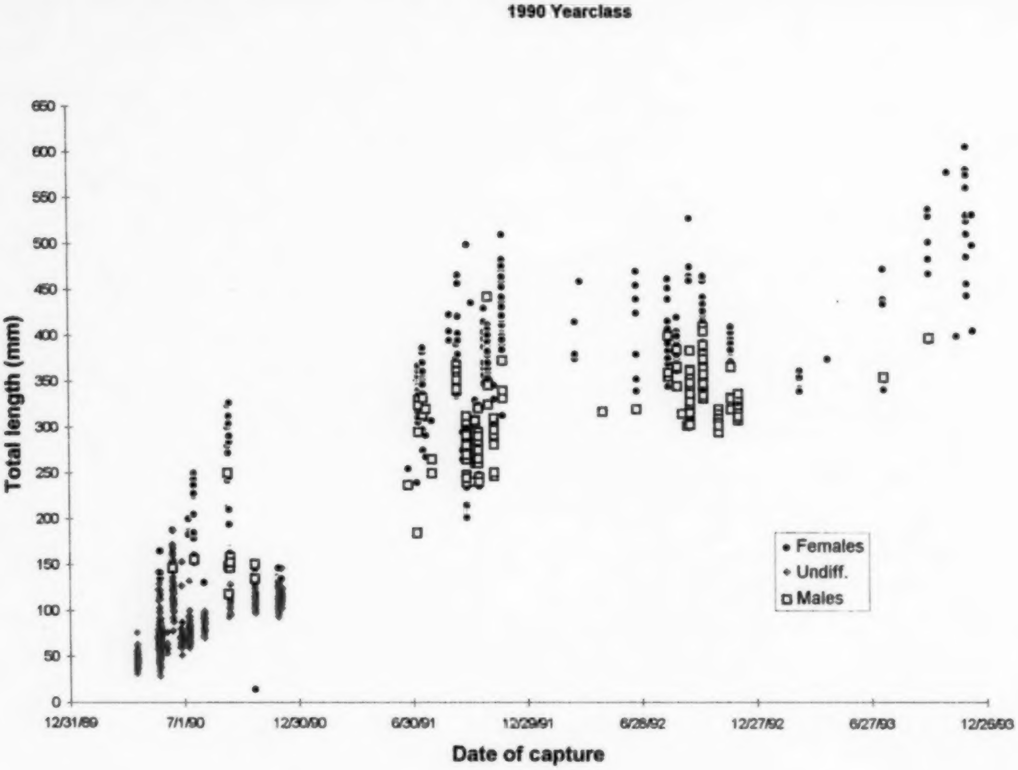
We randomly subsampled otoliths for daily growth estimates from nursery and sound collections made during 1990. Eleven to 30 sagittae (mean 19) were selected from monthly collections (samples archived from April to October), sectioned, polished, and aged. Details of our methods for preparation and interpretation of otolith microstructure for daily growth estimates were reported elsewhere (Fitzhugh and Rice 1995).

For diet analysis, data were available from nursery and sound survey collections made during 1988–1990. We recorded incidence of empty stomachs and whether contents could be identified if present (n = 1573 flounders). When identifiable prey items were present, we removed stomach contents and weighed and counted identifiable taxa. Often, individual fish prey could be identified to species. We used a binomial test to compare the proportions of male and female flounder stomachs with food (Hollander and Wolfe 1973) and stomach bolus weights were compared using a t-test.

Results

Southern flounder exhibited considerable variability in size at age for the 1990 year-class (Fig. 1). Some age-0 flounders were over 300 mm, overlapping older age-classes, and there was considerable overlap in size of 1-, 2-, and 3-year-old flounders. This overlap in size of age-0 and older flounders,

Fig. 1. Lengths of southern flounder by collection date for the 1990 year-class with samples representing age-0 to age-3 flounder.



reflecting rapid growth in some individuals, was most evident in September when juvenile fish were sampled for aging from deeper waters of Pamlico Sound as well as from shallow tributaries and embayments (Fig. 1).

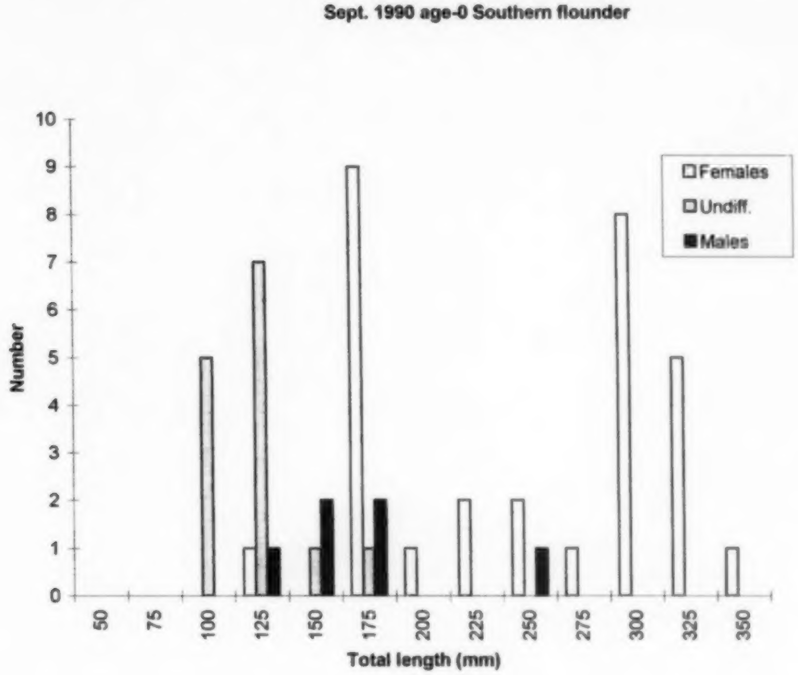
Sexually dimorphic differences in size-at-age were readily apparent by age 1 and 2, with females reaching larger sizes (Fig. 1). Any evidence of sexually divergent growth during the early juvenile phase was less clear as flounders were generally difficult to differentiate by sex below about 150 mm TL (Fig. 1). However, by fall 1990, we could detect a skewed size distribution with age-0 females reaching the largest sizes and broadly overlapping the size range of age-0 males (Fig. 1). When age-0 length data were pooled across depths, there was evidence of bimodality of size by September 1990 (Fig. 2). Males and undifferentiated fish contributed primarily to the lower mode (<200 mm), but females contributed to both the lower and upper mode (>275 mm). Based on length differences of fish for which we could determine sex, differential growth appeared to be primarily occurring among the females.

While dispersion of lengths was very apparent during the 1st year (Fig. 1), variance in length decreased with subsequent ages (Fig. 3). For the 1990 year-class, for which age-1 and older fish were sampled from the commercial fishery, it was evident that decreases in variance of length with age occurred for both males and females (Fig. 3). This decrease in variance of length with age suggests that compensating changes in factors such as growth and mortality occurs as the cohort ages.

To resolve whether the skewed size pattern, observed in age-0 flounder, was due to age differences in the first year, daily ages were determined from 130 flounders sampled in 1990. Using an age – ring count regression (Fitzhugh and Rice 1995), these flounders averaged 166 days in age, which we could detect within ±8 days (95% CI). We then tabulated the frequency of (BDs) from ring counts of sectioned otoliths. The back-calculated BDs from these age-0 juveniles ranged from November to May. The BD distribution was characterized by a protracted spawning season with a single mode and greatest frequency in late January (Fig. 4).

Variation in size increased with age in juveniles and we noted a bifurcation beginning at about 75–100 mm, corresponding to an age of about 150 days (Fig. 5). The resulting otolith-based growth rates, assuming linear growth trajectories, were quite variable. Observed growth rates of individuals ranged from 0.35 to 1.5 $\mathrm{mm \cdot d^{-1}}$ (0.65 ± 0.28 $\mathrm{mm \cdot d^{-1}}$; mean ± SD). To evaluate whether growth rate variation correlated with BD, we assigned relative BDs by arbitrarily dividing the cumulative BD distribution into thirds representing early, middle and late components (early BD <18 days, middle BD from 19 to 50 days, late BD >50 days; Fig. 4) based on the number of days from January 1 (day 0). There

Fig. 2. Length histogram of age-0 southern flounder sampled in September 1990 from the Pamlico Estuary.



was no clear pattern between relative BD and position (early, middle, late) on the growth curve (Fig. 5). At a given age, early spawned individuals were represented among all sizes, large and small. We reject the hypothesis that skewness or bimodality in flounder size distribution is determined by age.

Differences in otolith-determined growth rates as a function of sex is less clear but supports our observations of differences in lengths by sex across collection times. Our data were limited in the number of positive identifications of sex among juveniles, but females showed higher growth rates and a wider range in variation of growth. Eight males were aged with a mean growth rate of 0.7 ± 0.2 $\mathrm{mm \cdot d^{-1}}$, ranging from 0.6 to 1.2 $\mathrm{mm \cdot d^{-1}}$. Twenty-nine females were aged with a mean growth rate of 1.0 ± 0.3 $\mathrm{mm \cdot d^{-1}}$, ranging from 0.5 to 1.5 $\mathrm{mm \cdot d^{-1}}$.

Another factor thought to affect variation in growth among individuals is diet. In general, smaller flounders examined had a higher frequency of stomachs with prey items present (Table 1). Flounders less than 150 mm TL commonly fed on invertebrate prey, principally mysids, e.g., *Mysidopsis bigelowi* and *Neomysis americana*.

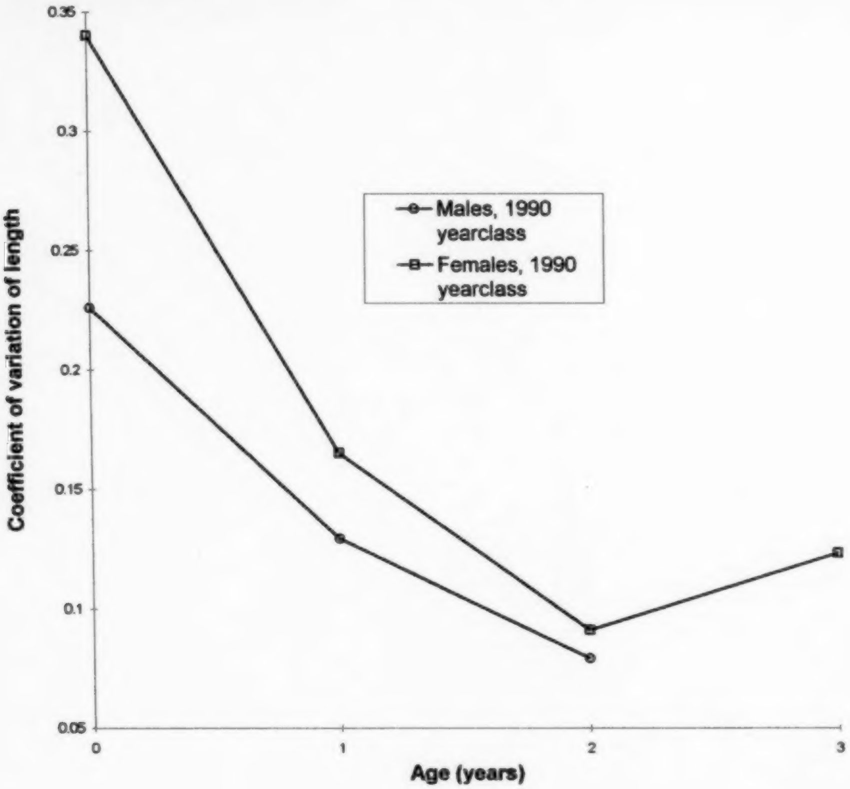
We observed an ontogenetic shift to fish prey as flounder size increased, with the onset of piscivory at about 70 mm (Fig. 6). As we examined progressively larger flounders, fish became more common among prey found in stomachs (Table 1, Fig. 6). The greatest increase in the proportion of flounders feeding on fish prey occurred between 110 mm (about 30% piscivorous) to 150 mm (about 65% piscivorous); sizes where sex of the fish could not be easily identified. When flounders reached 180 mm almost all stomachs with food included fish (Fig. 6). The middle size-classes examined (151–250 mm TL) generally exhibited the greatest percent weight and greatest frequency of fish prey (Table 1). The most common fish prey was bay anchovy (*Anchoa mitchilli*), followed by sciaenids, principally spot (*Leiostomus xanthurus*) and croaker (*Micropogonias undulatus*) (Table 1).

Since there was evidence that growth rates and sizes were more variable among females, we compared the diets of males and females within the 151–250 mm size-class, where sexes could be more readily identified, to see if there was evidence for variable food consumption. Within this size-class, we found that significantly more males had food in their stomachs (46% males : 32% females, $p = 0.0007$; Table 2); those males with food showed a significantly higher proportion of fish prey than females (92% males : 80% females, $p = 0.015$; Table 2), yet the mean weight of a food bolus for individuals that had eaten was not significantly different for males and females ($p = 0.7$; Table 2). These observations suggest that the consumption of fish prey was more variable among females than males.

Discussion

We found evidence for broad differential growth among age-0 southern flounder. Overall, female growth was faster and more variable resulting in a female size range completely overlapping that of males. There was some evidence that the age-0 female size distribution could become bimodal by September, and some age-0 individuals (no annulus present) were as large as 330 mm TL. This implies a higher

Fig. 3. Coefficient of variation of total length by age and sex for individuals of the 1990 year-class.



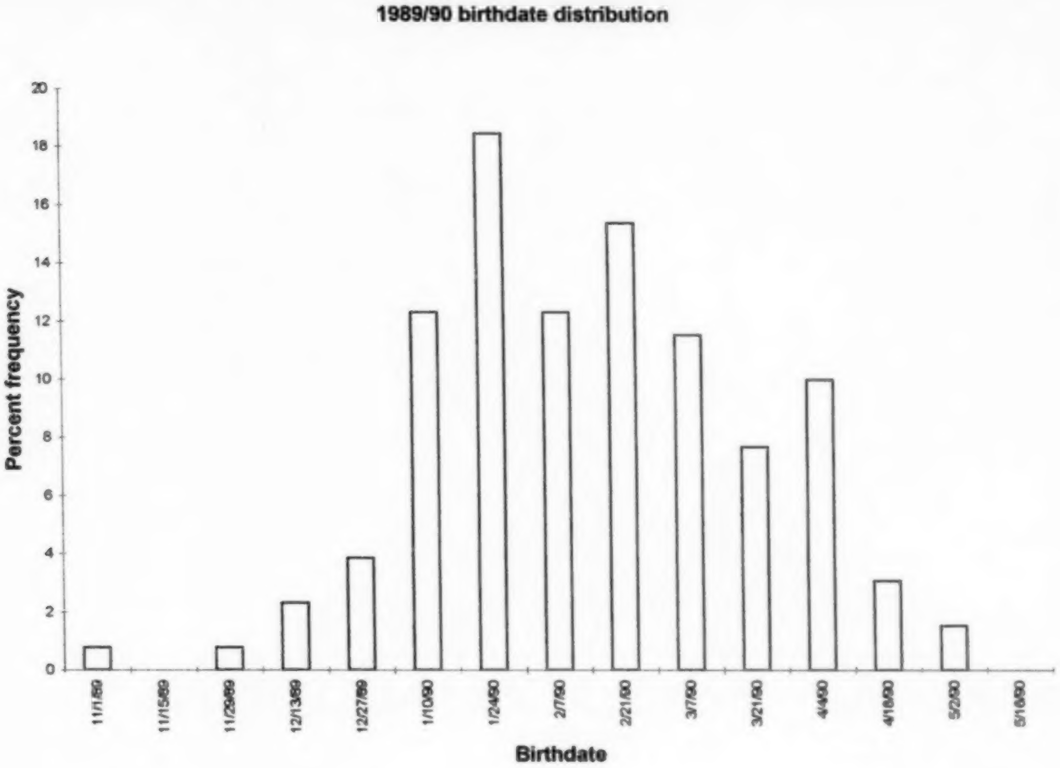
growth rate than has previously been recorded for southern flounder from field studies (cf. Powell and Schwartz 1977; Stokes 1977). Recently, juvenile summer flounder (*Paralichthys dentatus*) in New Jersey estuaries have been observed to reach 320 mm in length over their first summer, with sustained growth rates as high as 1.9 $\mathrm{mm \cdot d^{-1}}$ (Able et al. 1989; Szedlmayer et al. 1992). These findings indicate that high growth rates (>1 $\mathrm{mm \cdot d^{-1}}$) of paralichthid flounders can occur in the wild and imply that many flounders captured in North Carolina may be a year or more younger than previously presumed (cf. DeVries 1981).

The coefficient of variation for size at age of southern flounder declined with progressing age, which is a general pattern for many short-lived species (Bowker 1995). Therefore, the early dispersion of lengths are due to differences among individuals in initial size (i.e., due to BD) or initial growth trajectories. Size can be correlated to BD and in some fishes accounts for subsequent growth differences among components of a juvenile cohort (DeAngelis and Coutant 1982; Huston and DeAngelis 1987). For example, the largest juveniles have been observed to be the oldest in birth cohorts of fishes such as largemouth bass, *Micropterus salmoides*, (Miller and Storck 1984; Timmons et al. 1980; Isley and Noble 1987) and bluefish, *Pomatomus saltatrix* (Nyman and Conover 1988). Variation in length during the juvenile stage as a function of BD is more likely to be high in populations having different spawning groups and characterized by multiple modes in the spawning distribution (e.g., Atlantic croaker *Micropogonias undulatus*; Atlantic menhaden, *Brevoortia tyrannus* (Warlen and Burke 1990); and bluefish (Nyman and Conover 1988)). We did not find that earliest spawned southern flounder were consistently larger or faster growing among the 1990 year-class of juveniles that we aged.

Additionally, the initial distribution of flounder BDs was unimodal, ranging from November to May with a peak in late January. Our age results match the timing of larval arrival at Beaufort Inlet (Burke et al. 1991), if larval durations of 30–70 days are taken into account (Miller et al. 1991). Annual patterns of abundance for immigrating larvae also were unimodal and peaks of larvae (predominately in late metamorphosis) varied from mid-February to early March for 1986 – 1989 (Burke et al. 1991). This suggests that the size differences we detected in the juvenile stage for southern flounder were due to differential growth rates among individuals and were not merely a consequence of spawning date.

Sexual dimorphism in juvenile growth rates also could explain much of the size divergence. It was apparent, from the scatterplots of size at collection time, that females were exhibiting more variable growth than males. The daily aging data for juveniles, although limited by the number of positive identifications by sex, also supports the conclusion that growth rate is broadly overlapping yet greater for females even during

Fig. 4. Histogram of birthdate frequency by day of the year determined by otolith increment counts from age-0 flounders sampled in 1990.



the first year. However, our observations that growth rates of both sexes overlapped in range and that many of the females were also slow growers (<1 mm·d^{-1}) leads us to conclude that sexually dimorphic growth only partially explains the observed juvenile size differences as extreme skewing and possibly bimodal distribution of size occurred among females.

Our data suggest that diet changes were a major contributing factor for differential growth among age-0 flounder. The size-frequency distributions of 1990 age-0 flounders and the growth curve derived from sectioned otoliths revealed cohort splitting at about 75–100 mm. This size was correlated with onset of piscivory, e.g., about 20% of the juveniles sampled were piscivorous by 90 mm. Smaller juveniles continued eating invertebrates and had a relatively high frequency of stomachs with food present although the bolus size was small. Diets of larger flounder were characterized by an increased frequency of empty stomachs, increased occurrence of fish prey, and a larger food bolus. These observations are generally consistent for southern flounder diets in the Southeast Atlantic and Northern Gulf of Mexico (Fox and White 1969; Powell and Schwartz 1979; Overstreet and Heard 1982; Music and Pafford 1984; Wenner et al. 1990). Our observations are also consistent with other studies of piscivorous fishes, which show that onset of piscivory contributes to divergence in growth among juveniles (Forney 1966; Popova 1967; Timmons et al. 1980).

Our comparison of male and female diets, also limited to the number of positive identifications of sex among juveniles, may help explain our findings of more variable growth among females compared with males. Females were more likely to have empty stomachs, yet the average bolus weight was about the same as for similar-sized males (151–250 mm TL). This pattern may reflect that the switch to piscivory among females is characterized by greater variability in food consumption where ''winners'' and "losers" become more defined in terms of growth.

To explain the development of bimodality, as suggested by the September 1990 size histogram and previous findings by DeVries (1981) and Wenner et al. (1990), faster growers and slower growers must become well defined and separated in size frequency during the summer of their first year. Differential growth has been identified as a factor leading to bimodality in fish populations, but model simulations (Huston and DeAngelis 1987) suggest that the necessary factors for bimodality include sufficient variation in initial sizes, an increase in resource availability as size increases, and a corresponding decrease in resource availability for the smallest individuals.

Two ingredients for bimodality among southern flounder are evident: a unimodal, yet protracted, spawning distribution and size-related onset of piscivory. However, we lack direct evidence for any decrease in resource availability for

Table 1. Summary of food items from southern flounder stomachs tabulated by flounder size class (mm TL).

	<101 mm	101–150 mm	151–200 mm	201–250 mm	251–300 mm	301–350 mm	351–400 mm
Total stomachs	563	589	196	138	58	24	5
% with food	(66)	(47)	(39)	(42)	(46)	(21)	(20)
% empty	(18)	(40)	(47)	(51)	(45)	(54)	(40)
% unrecognizable	(26)	(13)	(14)	(7)	(9)	(25)	(40)
Total no. of food items	2706	3360	187	86	72	7	1
Total weight (g) of food items	36.4	111.2	79.3	121.5	72.1	24.9	2.9
Bay anchovy							
Occurrence	2 (0.6)	19 (6.9)	18 (27.7)	25 (43.1)	7 (25.9)	2 (40)	
Weight (g)	0.6 (1.6)	17.7 (15.9)	19 (24)	36.8 (30.3)	10.9 (15.1)	4.4 (17.7)	
Frequency	2 (0.1)	21 (0.6)	24 (12.8)	43 (50)	11 (15.3)	3 (42.9)	
Sciaenids							
Occurrence	7 (2.2)	26 (9.5)	19 (25)	15 (25.9)	6 (22.2)	1 (20)	
Weight (g)	5.7 (15.7)	35.3 (31.7)	41.9 (52.8)	64.6 (53.2)	33.5 (46.5)	20 (80.3)	
Frequency	9 (0.3)	28 (0.8)	20 (10.7)	18 (20.9)	8 (11.1)	3 (42.9)	
Other fish prey							
Occurrence	18 (5.7)	56 (20.4)	18 (23.7)	16 (27.6)	8 (29.6)	1 (20)	1 (100)
Weight (g)	7.0 (19.2)	40.4 (36.3)	15.9 (20.1)	19 (15.6)	19.1 (26.5)	0.5 (2)	2.9 (100)
Frequency	19 (0.7)	61 (1.8)	19 (10.2)	17 (19.8)	10 (13.9)	1 (14.3)	1 (100)
Mysids							
Occurrence	255 (81)	152 (55.3)	12 (15.8)	3 (5.2)	1 (3.7)		
Weight (g)	8.6 (23.6)	16.9 (15.2)	2.5 (3.2)	0.5 (0.4)	0.9 (1.2)		
Frequency	2543 (94)	3221 (96)	114 (61)	4 (47)	40 (55.6)		
Other invertebrate prey							
Occurrence	22 (7)	11 (4)	3 (3.9)	2 (3.4)	2 (7.4)		
Weight (g)	14.4 (39.6)	0.8 (0.7)	0.2 (0.3)	0.6 (0.5)	7.8 (10.8)		
Frequency	133 (4.9)	29 (0.8)	10 (5.4)	4 (4.7)	3 (4.2)		

Note: Column percentages are shown in parentheses. Occurrence refers to the number of flounder stomachs (with food) each prey category occurred in and frequency is the number of individual prey items recovered within a flounder size-class.

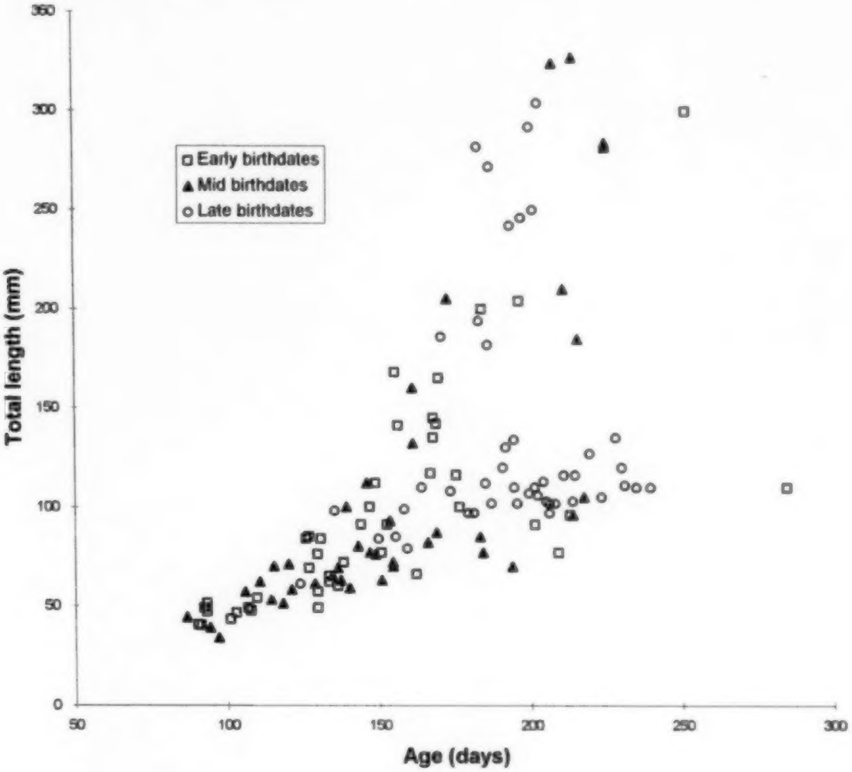
Table 2. Percent piscivory (for males and females with food in their stomachs) and mean weight of food consumed by southern flounder in the size class 151–250 mm TL.

	No of flounders with food in stomachs (% of total in size class)	No. piscivorous (percent)	Mean bolus weight (g)	SE
Males	50 (46)	46 (92)	1.9	0.33
Females	59 (32)	47 (80)	2.0	0.27

the smallest individuals during the first summer. This must occur for a bimodal size distribution to become pronounced. Reduced growth among smallest individuals is usually attributed to reduced food availability as a function of size (Huston and DeAngelis 1987), and this is a possible factor in our system as well. For example, we noted that a large component of juvenile flounders (including females as well as undifferentiated juveniles and males) sampled in shallow-water trawls only reached the size where the proportion of piscivorous flounders would be expected to accelerate (100–150 mm TL) by fall (Sepember-December 1989 and 1990). However, by middle to late summer, common preferred prey, such as bay anchovies, spot, and croaker, may have grown to a size where they become invulnerable to these smaller sized flounders. Experiments with southern flounder and spot demonstrate that fast growth of prey (spot) relative to predators (flounders) can result in a size refuge, and this generally occurs when spot are greater than 40% of flounder length (Rice et al. 1993). Additionally, spot and croaker reach peak densities in April–May in creeks and bays of the Pamlico estuary and abundances decline steadily through the summer and fall (Ross and Epperly 1985). Therefore, it is likely that availability of fish prey declines for small flounders (i.e., <150 mm) both because of increasing prey size and decreasing abundance throughout the summer months.

We cannot reject the possibility that other factors besides

Fig. 5. Otolith ring count (age in days) versus total length for age-0 flounders sampled in 1990. Symbols represent birthdates falling early, middle, or late in the season relative to the cohort birthdate distribution (see text).



declining availability of fish prey could inhibit growth in shallow habitats. The Pamlico and Neuse rivers can experience sustained hypoxic conditions (<5 ppm) during summer months (Garrett and Bales 1991; Stanley and Nixon 1992). Juvenile southern flounder avoid areas of depleted oxygen under experimental conditions (below 3.7 ppm; Deubler and Posner 1963). While low dissolved oxygen can occur in deeper waters because of salinity stratification, shallow estuarine areas in this system are also susceptible to low dissolved oxygen in summer months because of high temperatures and biological oxygen demand (Kirby-Smith and Costlow 1989; Paerl et al. 1995). Low-oxygen conditions, together with elevated summer temperatures, can retard growth rates in certain fishes (Brett 1979). Additionally, juvenile growth may be sensitive to fluctuating oxygen levels commonly found in shallow estuaries, as well as to constant low oxygen levels (Bejda et al. 1992).

We suggest that the initial spreading of growth rates arises through size-related differences in onset of piscivory (i.e., as proposed by DeAngelis and Coutant (1982) and Huston and DeAngelis (1987). We found evidence for bimodality of size by September 1990 with undifferentiated juveniles, males, and females in a lower mode and primarily females in an upper mode. However, we found no evidence for bimodality due to bimodal reproduction over an extended spawning season as has been proposed for other species (Huston and DeAngelis 1987). Differential feeding opportunities and the onset of piscivory provide a plausible explanation for bimodal growth that may occur, particularly among females. We could not test for factors that may retard growth of the smallest sized individuals during summer months. For example, the possibility exists that growth of smallest flounders may be limited by environmental conditions such as low oxygen level coupled with high temperature in shallow water or the lack of appropriately sized fish prey during the summer. A more complete evaluation of the shallow-water residence time of individuals as a function of size also needs to be completed before this hypothesis can be examined further.

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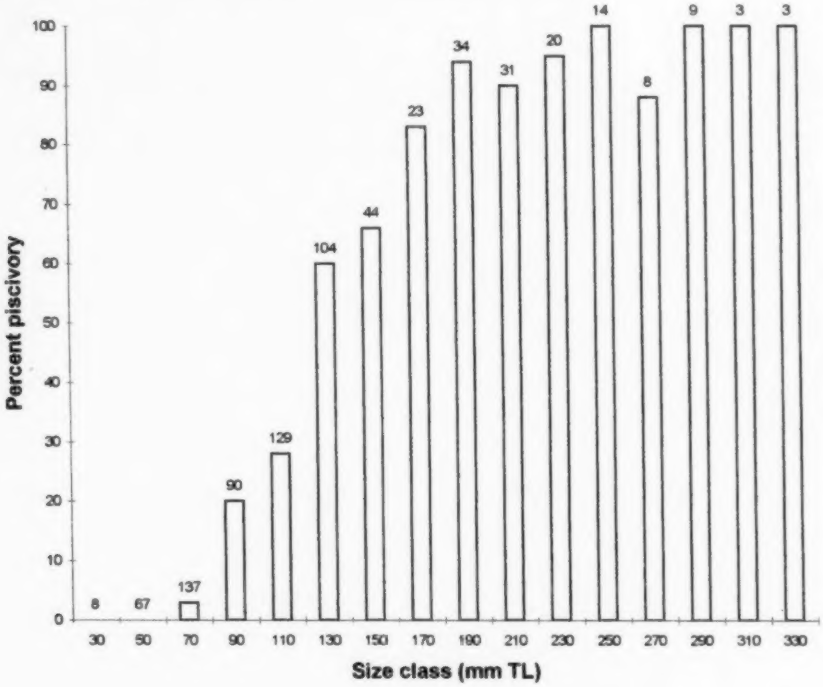
Fig. 6. Percentage of flounders with fish in their diet relative to all flounders in each size-class with identifiable food in their stomach. Number of flounders with food in stomachs is indicated above each bar.



Atmospheric Administration, and the University of North Carolina Sea Grant Program under Grant No. NA90AA-D-SG062; by the Electric Power Research Institute Fellowship Program in Population Dynamics under Grant No. 90–02; and by Grant No. NA16RG0492 from the Coastal Ocean Program of the National Oceanic and Atmospheric Administration to the North Carolina Sea Grant Program. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies. This study represents a portion of a dissertation submitted in partial fulfillment for the Ph.D. degree by G.R.F. at North Carolina State University.

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Microcystin-LR concentration in aquatic food web compartments from lakes of varying trophic status

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Abstract: Microcystin-LR (MC-LR) concentrations were examined in water, phytoplankton, invertebrates, and two fishes for up to 3 years in four central Alberta lakes spanning a trophic gradient in total phosphorus from 15 to over 500 $\mu g \cdot L^{-1}$ in epilimnetic waters. MC-LR was not detected by HPLC in phytoplankton from an oligo-mesotrophic lake, was less than 150 ng cellular toxin$\cdot L^{-1}$ of lake water in a eutrophic–hypereutrophic lake, and was up to 6000 and 11 000 ng$\cdot L^{-1}$ in two hypereutrophic lakes. MC-LR in phytoplankton was strongly correlated with the abundance of the cyanobacterium *Microcystis aeruginosa*, and with aqueous microcystin concentration, determined by protein phosphatase bioassay ($r = 0.83$). MC-LR was also detected in zooplankton (up to 67 $\mu g \cdot g^{-1}$ of biomass) and MC-LR concentration in zooplankton and phytoplankton were correlated ($r = 0.69$). Although nine groups of macroinvertebrates were analyzed, MC-LR was only detected in gastropods (up to 120 $\mu g \cdot g^{-1}$). MC-LR appears to be transferred to invertebrates through grazing activity. MC-LR was not detected in the livers of northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) collected from one lake containing toxin-producing phytoplankton. Accumulation of MC-LR in aquatic food webs appears to occur in the primary consumer with probable transfer of the toxin to higher trophic levels.

Résumé : Pendant une période atteignant une durée de 3 ans, on a mesuré les concentrations de microcystine-LR (MC-LR) dans l'eau et le phytoplancton ainsi que chez les invertébrés et chez deux espèces de poissons de 4 lacs du centre de l'Alberta où le gradient trophique de phosphore total épilimnétique allait de 15 à plus de 500 $\mu g \cdot L^{-1}$. La HPLC du phytoplancton du lac oligomésotrophe n'a pas mis en évidence de MC-LR; la concentration de la toxine cellulaire était inférieure à 150 ng$\cdot L^{-1}$ d'eau dans le lac eutrophe–hypereutrophe et elle atteignait 6000 et 11 000 ng$\cdot L^{-1}$ dans les deux hypereutrophes. Par ailleurs, on a noté une forte corrélation entre la concentration de MC-LR phytoplanctonique et l'abondance de la cyanobactérie *Microcystis aeruginosa* et la concentration de microcystine aqueuse, dosée avec une protéine-phosphatase ($r = 0{,}83$). On a aussi détecté de la MC-LR chez le zooplancton (jusqu'à 67 $\mu g \cdot g^{-1}$ de biomasse) et observé une corrélation entre les concentrations de MC-LR zooplanctonique et phytoplanctonique ($r = 0{,}69$). On a analysé neuf groupes de macroinvertébrés, mais la MC-LR n'a été détecté que chez les gastéropodes (jusqu'à 120 $\mu g \cdot g^{-1}$). La MC-LR semble passer aux invertébrés par le broutage. On n'en a pas détecté dans le foie du broche (*Esox lucius*) et du meunier noir (*Catostomus commersoni*) prélevés dans l'un des lacs où le phytoplancton est producteur de toxine. La bioconcentation de MC-LR dans les réseaux trophiques aquatiques semble survenir au niveau des consommateurs primaires et il y a probablement transfert de la toxine aux niveaux trophiques plus élevés.
[Traduit par la Rédaction]

Introduction

Microcystins are hepatotoxic compounds produced primarily by the cyanobacterium *Microcystis aeruginosa* (Namikoshi et al. 1992; Kotak et al. 1993, 1995). Microcystin production by cyanobacteria is likely a common occurrence in eutrophic and hypereutrophic lakes in western Canada. Kotak (1995) recorded the toxin in 24 of 28 lakes surveyed from 1990 to 1994 in central and northern Alberta, with 360 of the 490 phytoplankton samples collected containing detectable concentrations of microcystin-LR (MC-LR). Although more than 50 microcystins have been structurally characterized, MC-LR is the most common analogue in Alberta lakes (Boland et al. 1993; Craig et al. 1993; Kotak et al. 1993). It is also one of the most toxic of the microcystins, having an LD_{50} of 50 $\mu g \cdot kg^{-1}$ by intraperitoneal injection in mice (Carmichael 1992).

Much of the research on the toxicity of microcystins has traditionally focused on laboratory mammals (e.g., rats, mice). More recently, studies have evaluated the relationship between toxic cyanobacteria and other components of aquatic food webs. However, most of these studies examined only acute toxicity, in either zooplankton (DeMott et al. 1991; Rothhaupt 1991; Gilbert 1994; Jungmann and Benndorf 1994; Reinikainen et al. 1994) or fish (Phillips et al. 1985; Sugaya et al. 1990; Rabergh et al. 1991; Kotak et al. 1996). Only one study has evaluated whether microcystins are accumulated by natural populations of aquatic invertebrates (Watanabe et al. 1992) in freshwater lakes. Recently, MC-LR has also been detected in marine organisms. Chen et al. (1993) reported MC-LR in mussels collected from coastal waters of British Columbia, and Andersen et al. (1993) documented detectable concentrations of MC-LR in aquatic invertebrates fouling Atlantic salmon (*Salmo salar*) net pens, as

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well as in the livers of salmon with severe liver disease (Kent et al. 1988; Kent 1990). The source (producer) of MC-LR in the marine environment, however, is currently unknown. Our study reports the concentration of MC-LR in five compartments (water, phytoplankton, micro- and macro-invertebrates, and to a limited extent, fish) from up to four lakes in central Alberta, ranging in trophic status from oligo-mesotrophic to hypereutrophic. MC-LR concentrations were then related to physical and biological characteristics of the lakes.

Materials and methods

Study lakes

The four lakes chosen for study are located in central Alberta, Canada: Coal, Driedmeat, Little Beaver, and Narrow lakes. Coal and Little Beaver lakes were sampled every 2 weeks from May to the end of September in 1992 and May to the end of August in 1993 and 1994. Driedmeat Lake was sampled similarly, although sampling in 1993 extended until the end of September. Narrow Lake was sampled only in 1994: on 13 May, 14 June, 11 July, and 20 September. The lakes chosen differed in trophic status and, thus, in the potential for MC-LR production. Historically, Narrow Lake is oligo-mesotrophic, Coal Lake is eutrophic, and Driedmeat and Little Beaver lakes are hypereutrophic based on summer total phosphorus and chlorophyll *a* concentrations in the euphotic zone (Mitchell and Prepas 1990; Kotak et al. 1993).

Water quality

On each sampling date an integrated water sample was collected from the surface of the water to a depth of 2 m (except for in Little Beaver Lake where water was collected from the surface to a depth of 1 m) at four sites with Tygon tubing fitted with a one-way valve. These samples are referred to as epilimnetic. Water from these four sites was combined prior to analyses. Water was analyzed for total phosphorus by colorimetric methods after potassium persulfate digestion (Menzel and Corwin 1965, as modified by Prepas and Rigler 1982), although large particles (>250 μm) were not filtered out. Conductivity was measured on a Radiometer Analytical Model CDM83 conductance meter. Alkalinity (as milligrams $CaCO_3$ per litre) was determined on a Mettler DL21 autotitrator (APHA, AWWA, and WPCF 1992). A small subsample (10 mL) of the water was filtered through a pre-rinsed, 0.45-μm Millipore HA filter and frozen for subsequent aqueous microcystin analysis. Phytoplankton biomass (estimated as chlorophyll *a*) was determined from water samples that were filtered onto Whatman GF/C filters, extracted in 95% ethanol, and measured spectrophotometrically (Bergmann and Peters 1980). Phytoplankton were identified and enumerated on an inverted microscope (Prescott 1978). *Microcystis aeruginosa* abundance (cells per millilitre) is reported. Secchi disk depth and water temperature were recorded at one central site in each lake. Surface water temperature was recorded with a YSI Model 51 temperature – O_2 meter. Total phosphorus, conductivity, and Secchi disk depth for 1994 are reported as summer means from May to September (±SE). Mean summer alkalinity (±SE) in Coal, Driedmeat, and Little Beaver lakes are reported for 1993.

Zooplankton

In 1992 and 1993 in Coal, Driedmeat, and Little Beaver lakes and in 1994 in Narrow Lake, epilimnetic zooplankton samples were collected with a 243-μm mesh plankton net. One vertical haul was made at each of four sites per lake and the four samples combined for zooplankton identification. Samples were preserved in 10% buffered formalin and zooplankton were identified and enumerated with a dissecting microscope. Identification was based on descriptions in Pennak (1978). Abundance of each species was then expressed as a percentage of the total abundance in the sample. Zooplankton for microcystin-LR (MC-LR) analysis were collected as described above. The zooplankton samples (which also contained phytoplankton) were then resuspended in 1 L of lake water. Upon arrival at the laboratory, the plankton sample was gently bubbled with CO_2 to anesthetize the zooplankton. The sample was then tightly capped and the zooplankton settled to the bottom of the jar while the cyanobacteria rose to the surface. The cyanobacteria that accumulated along the surface and most of the water were siphoned off, avoiding removal of the zooplankton on the bottom. The remaining zooplankton and phytoplankton were resuspended in distilled water and the process repeated. The samples contained only zooplankton and groups of phytoplankton that settled out quickly (e.g., diatoms). Microscopic examination of the processed sample revealed no detectable *M. aeruginosa*, the species responsible for MC-LR production in Alberta lakes (Kotak et al. 1993, 1995). Thus, any MC-LR detected in these samples was presumably from zooplankton tissue or gut contents. The sample was then frozen and freeze-dried. On several sampling dates, zooplankton biomass was insufficient for MC-LR analysis. As a result, only 68 of 99 zooplankton samples collected were analyzed for MC-LR.

Phytoplankton

In all years, phytoplankton were collected for MC-LR analysis with a 64-μm mesh plankton net at the same locations and in a similar manner as that for zooplankton. The samples collected at the four sites in each lake were combined and later freeze-dried. These samples contained both phytoplankton and zooplankton. To determine the concentration of MC-LR in the phytoplankton alone, MC-LR concentration detected in the separate zooplankton sample was subtracted from the MC-LR concentration detected in the sample containing both phytoplankton and zooplankton. The correction for the presence of zooplankton was not always possible as there were 99 phytoplankton samples but only 68 zooplankton samples.

Macroinvertebrates

In 1994, a suite of aquatic macroinvertebrates was collected from each of the four lakes and analyzed for MC-LR. These included gastropods (*Lymnaea stagnalis*, *Physa gyrina*, *Helisoma trivolvis*), dragonfly (*Ashna* sp.), and damselfly (*Lestes* sp.) larvae, water boatmen (Family Corixidae), backswimmers (*Notonecta* sp.), predacious water beetles (*Dytiscus* sp.), scuds (*Gammarus lacustris*), and leeches (Subclass Hirudinea). Identifications were based on descriptions given in Clifford (1993). These invertebrates were collected with a dip net from the shoreline or from the littoral zone from the side of the boat. In addition, surface sediments were collected from four sites in each lake with an Eckmann dredge and the four samples were combined. Mud was sifted through a 500-μm sieve and chironomids (*Chironomus* spp.) were rinsed and saved for MC-LR analysis. All invertebrates (including zooplankton) were frozen, freeze-dried, and then homogenized with a mortar and pestle.

Fish

On 29 and 30 August 1993, northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) were collected from Driedmeat Lake with a combination of gill and seine nets. Driedmeat Lake was chosen as the site for fish collection because we felt the fish had been exposed to high concentrations of MC-LR in the phytoplankton during the previous month. The eight northern pike collected ranged in size from 38 to 46 cm (fork length) and weighed from 445 to 814 g. The 23 suckers collected ranged in size from 32 to 42 cm and weighed from 584 to 1302 g. The fish were immediately frozen and later thawed to remove their livers for MC-LR analysis. The fish were weighed (wet mass), and a limited examination of the gut contents of the fish was made. Livers were freeze-dried prior to MC-LR analysis.

Microcystin analyses

MC-LR in phytoplankton and aquatic invertebrates was quantified

Table 1. Water quality and morphometric characteristics of the study lakes.

Variable	Lake: Narrow	Coal	Little Beaver	Driedmeat
Total Phosphorus ($\mu g \cdot L^{-1}$)	15 (2)	71 (17)	207 (26)	530 (119)
Secchi depth (m)	7.2 (0.5)	1.3 (0.1)	0.8 (0.2)	1.1 (0.3)
Conductivity ($\mu S \cdot cm^{-1}$)	231 (41)	474 (12)	826 (65)	574 (14)
Alkalinity ($mg \cdot L^{-1}$ $CaCO_3$)*	155 (1)	196 (6)	332 (11)	210 (10)
Surface area (km^2)	1.1	11	0.75	17
Maximum depth (m)	39	5.5	2.0	3.7

Note: Water quality parameters (total phosphorus, Secchi disk depth, conductivity, and alkalinity) represent seasonal means (May to September, with SE given in parentheses) for 1994 and are based on samples collected from the surface of the water to a depth of 2 m (surface to 1 m for Little Beaver Lake), except where indicated. Lake morphometry data were obtained from Mitchell and Prepas (1990) and Kotak et al. (1993).

*Alkalinity in Narrow Lake is for 1983 (Mitchell and Prepas 1990) and for 1993 in Coal, Little Beaver, and Driedmeat lakes.

by reverse phase high performance liquid chromatography (HPLC). Phytoplankton and zooplankton, and macroinvertebrate subsamples (100 and 500 mg freeze-dried weight, respectively) were sonicated and extracted in 5% acetic acid overnight. The extract was then filtered through Whatman GF/A filters and the microcystins purified and concentrated on preconditioned Supelco LC-18 cartridges. Microcystins were eluted from the cartridge in 5 mL 100% methanol. The methanol was evaporated in a Reactivap heater and the sample resuspended in 2 mL of 10 mM ammonium acetate : acetonitrile (75%:25%, v:v).

Over the 3 years of this program, three different HPLC separation methods were utilized on two separate HPLC systems. In 1992, phytoplankton were analyzed on a HPLC consisting of Waters 6000A pumps with a Hewlett Packard HP 1040A diode array detector and HP85 data processor. Samples were run under isocratic conditions at a flow rate of 1 $mL \cdot min^{-1}$. The mobile phase consisted of 10 mM ammonium acetate and acetonitrile (74:26%, v:v). In 1993 and 1994, phytoplankton were analyzed on a Waters LC Module I HPLC with a model 746 data module. For both HPLCs, MC-LR detection was based on UV absorbance at 238 nm. In 1993, samples were run under isocratic conditions, at a flow rate of 1 $mL \cdot min^{-1}$, and with a mobile phase consisting of 10 mM ammonium acetate: acetonitrile (76:24%, v:v). In 1994, samples were run under linear gradient conditions (20–30% acetonitrile in 10 mM ammonium acetate over 10 min, then at 20% from 10 min until the end of the run, (i.e., 30 min). Flow rate was 1 $mL \cdot min^{-1}$. A comparison of the two HPLCs and the three separation methods (two isocratic, one gradient) indicated no significant difference in the quantity of MC-LR detected, although the gradient method gave much better separation of MC-LR away from other interfering peaks (Kotak 1995). MC-LR concentration in phytoplankton was first calculated as micrograms per gram of phytoplankton biomass based on MC-LR calibration curves. MC-LR was then re-expressed volumetrically, as nanograms cellular toxin per litre (Kotak et al. 1995). This volumetric expression represented MC-LR in the phytoplankton cells per litre of lake water, not as dissolved toxin.

All invertebrate samples (zooplankton and macroinvertebrates) were analyzed for MC-LR on the latter HPLC system under the gradient conditions described above. MC-LR concentrations in the invertebrates are reported as micrograms per gram of biomass. In addition, the protein phosphatase (PP1c) assay (Luu et al. 1993) was also utilized to quantify total microcystin concentration in the zooplankton samples as well as total aqueous (dissolved) microcystin concentration in lake water. This gave an indication of the quantity of MC-LR and other microcystin analogues in the samples. The assay results are expressed as total microcystin concentration (as there may be more than 50 microcystins present in a sample) in MC-LR equivalent mass units. The detection limit of the PP1c assay was less than 10 pg MC-LR equivalents while the detection limit for MC-LR by HPLC was 10–20 ng for a 20-μL injection. For the PP1c assay, subsamples (100–500 mg) of zooplankton were extracted as described above for HPLC, except that the extraction solvent was 100% methanol and microcystins were not preconcentrated on LC-18 cartridges. Raw lake water samples were analyzed without prior treatment.

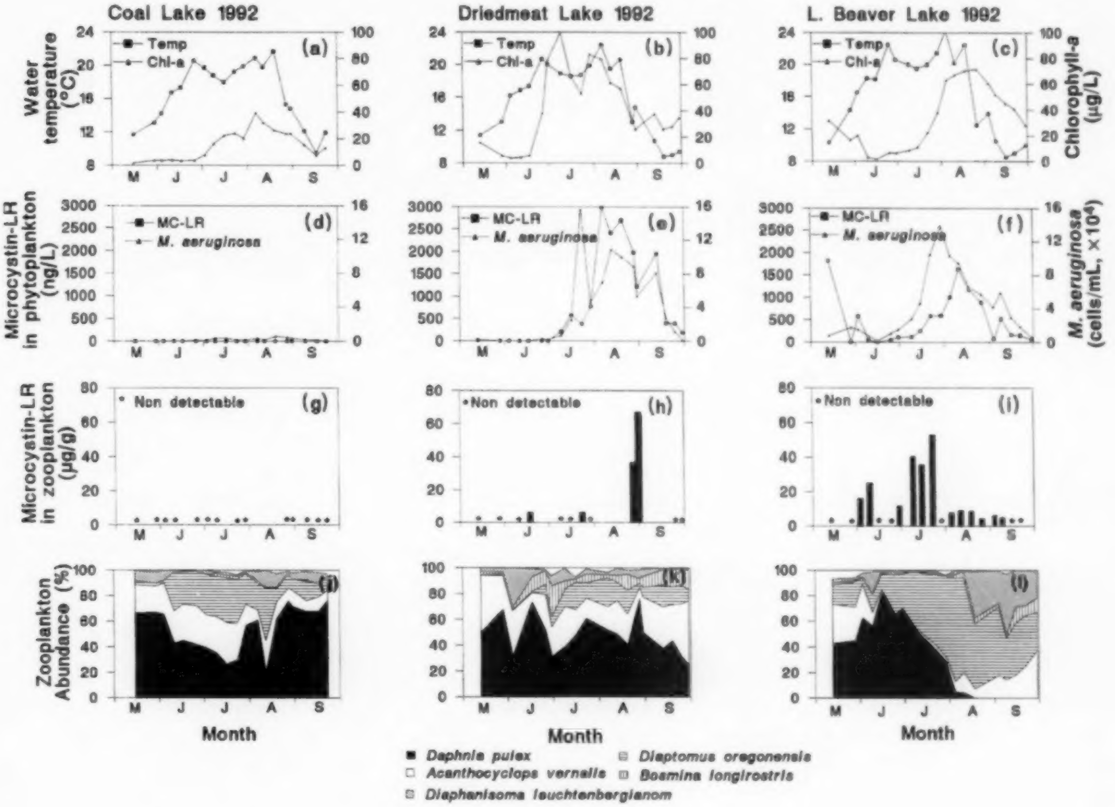
Livers from the fish collected from Driedmeat Lake were homogenized with a mortar and pestle and extracted in 100 mL, of 100% methanol. The methanol was evaporated to approximately 10 mL, and 400 mL of deionized distilled water was added. The sample was then passed through pre-conditioned Supelco C-18 cartridges and the sample eluted in 100% methanol as described earlier. The extract was then filtered through a 0.45-μm Millipore HV filter and injected onto a Waters LC Module I HPLC and run under gradient conditions (described above).

To confirm the presence of MC-LR (and possibly other microcystins) in the various compartments sampled, one sample of phytoplankton (collected from Driedmeat Lake on 17 August 1994), zooplankton (collected from Driedmeat Lake on 11 August 1993), gastropod (*Physa gyrina* collected from Driedmeat Lake on 17 August 1994), and northern pike liver and sucker liver (collected from Driedmeat Lake on 29 and 30 August 1993) were fractionated by HPLC and each fraction analyzed for PP1c inhibition. Samples were run on a Waters LC Module I HPLC under the gradient conditions previously described. Between five and eight (depending on the sample) 0.2- to 1.0-min fractions (i.e., 0.2–1.0 mL fractions) were collected over the 20-min runs. One fraction represented the retention time window where MC-LR was expected to elute from the column, and the other fractions represented other unidentified peaks and also areas in the chromatograms where no peaks occurred. The fractions were then analyzed for the ability to inhibit PP1c. Additionally, the presence of MC-LR in two invertebrate samples (the zooplankton and gastropod identified above) was confirmed by spiking the samples with a known amount of MC-LR standard.

Statistical analysis

MC-LR concentrations in water, phytoplankton, zooplankton and gastropods are also reported as summer means (May to September) (±SE) for the years in which the compartments were sampled.

Fig. 1. Seasonal changes in surface water temperature and chlorophyll *a* (*a*–*c*), *Microcystis aeruginosa* abundance and MC-LR concentration in the phytoplankton (*d*–*f*), MC-LR concentration in the zooplankton community (*g*–*i*), and zooplankton species composition (*j*–*l*) for Coal (left panels), Driedmeat (middle panels), and Little Beaver (right panels) lakes for 1992. Samples were collected from the 0- to 2-m stratum of the water column in Coal and Driedmeat lakes, and from the 0- to 1-m stratum in Little Beaver Lake. MC-LR was quantified by HPLC.



Correlation analyses were performed on log-transformed data with SPSS for Windows (version 6.0) statistical software.

Results

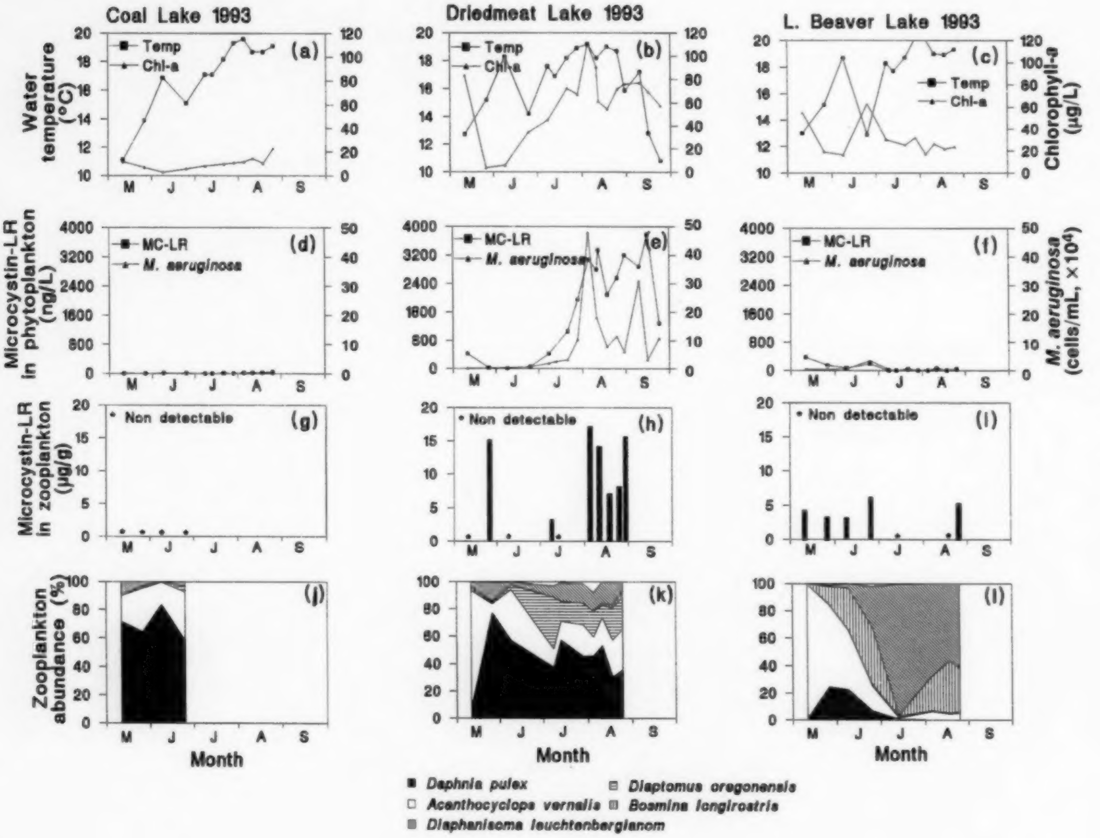
Water quality

In 1994, the only year for which data were collected from all four lakes for this study, Narrow Lake was classified as oligomesotrophic based on epilimnetic total phosphorus concentration, and Coal, Driedmeat, and Little Beaver lakes were hypereutrophic but differed substantially from each other (Table 1). Narrow Lake had the most transparent water (Secchi depth averaged 7.2 m in 1994), while Coal, Driedmeat, and Little Beaver lakes were much less transparent, with an average Secchi depth less than 1.3, 1.1, and 0.8 m, respectively. The water in the four lakes ranged from fresh to slightly saline in 1994, and all four lakes contained hard water (Table 1). The hypereutrophic lakes are all shallow (<6 m) and well mixed by the wind while Narrow Lake is deep (maximum depth of 38 m) and thermally stratified during summer.

The three hypereutrophic lakes contained the same pool of cyanobacterial genera over the 3 years and differed markedly from Narrow Lake. From 1992 to 1994 the dominant phytoplankton taxa in the three hypereutrophic lakes was usually cyanobacteria: *Microcystis aeruginosa*, *Aphanizomenon flos-aquae*, *Gomphosphaeria* sp., *Anabaena flos-aquae*, and *Phormidium* sp. (Kotak et al. 1995; Lam 1994). The phytoplankton community in Narrow Lake during 1994 included chlorophytes (*Clamydomonas* sp.), cryptophytes (*Rhodomonas* sp.), and chrysophytes (*Ochromonas* sp.).

Chlorophyll *a* concentrations differed dramatically among study lakes. On the four dates in 1994 when we sampled Narrow Lake, chlorophyll *a* was less than 2 $\mu g \cdot L^{-1}$. Chlorophyll *a* in the three hypereutrophic lakes was lowest in Coal Lake, was fairly consistent among years, and peaked in August (Figs. 1*a*, 2*a*, and 3*a*). Chlorophyll *a* varied most among years in Little Beaver Lake (Figs. 1*c*, 2*c*, and 3*c*), with mean summer chlorophyll *a* of 33 and 30 $\mu g \cdot L^{-1}$ in 1992 and 1993, respectively, and 67 $\mu g \cdot L^{-1}$ in 1994. On 17 August 1994 it exceeded 150 $\mu g \cdot L^{-1}$. Driedmeat Lake generally had the highest chlorophyll *a* concentration of the lakes studied over the 3 years: mean summer concentration of

Fig. 2. Seasonal changes in surface water temperature and chlorophyll *a* (*a–c*), *Microcystis aeruginosa* abundance and MC-LR concentration in the phytoplankton (*d–f*), MC-LR concentration in zooplankton community (*g–i*), and zooplankton species composition (*j–l*) for Coal (left panels), Driedmeat (middle panels), and Little Beaver (right panels) lakes for 1993. Samples were collected from the 0- to 2-m stratum of the water column in Coal and Driedmeat lakes, and from the 0- to 1-m stratum in Little Beaver Lake. MC-LR was quantified by HPLC.



51 µg·L^{-1} for all 3 years, with maxima for chlorophyll *a* over 100 µg·L^{-1} in July and August of 1992 and 1993 (Figs. 1*b* and 2*b*).

Microcystin-LR in phytoplankton

MC-LR concentration in the phytoplankton was highest in the two most hypereutrophic lakes (Driedmeat and Little Beaver). MC-LR concentration in the phytoplankton from Driedmeat Lake was highest from August to early September (1212 – 2987 ng·L^{-1} in 1992 and 2093 – 3780 ng·L^{-1} in 1993; Figs. 1*e* and 2*e*) and highest near the end of August in 1994 (5778 to 6069 ng·L^{-1} Fig. 3*e*). Peak MC-LR concentration in Driedmeat Lake phytoplankton corresponded to periods when *M. aeruginosa* abundance was at its seasonal high (usually greater than 200 000 cells·mL^{-1} in August; Figs. 1*e*, 2*e*, and 3*e*). Both *M. aeruginosa* abundance and MC-LR concentration in the phytoplankton also differed substantially among the 3 years of study in the two lakes. For example, in Little Beaver Lake, mean summer *M. aeruginosa* abundance and MC-LR concentration in phytoplankton was 43 024 cells·mL^{-1} and 488 ng·L^{-1}, respectively, in 1992, decreased 9- and 6-fold, respectively, in 1993, and in 1994 increased almost 15- and 57-fold relative to 1993 (Figs. 2*f* and 3*f*). The highest MC-LR concentration measured during the 3 years was in phytoplankton from Little Beaver Lake (11 216 ng·L^{-1}) on 17 August 1994. Both *M. aeruginosa* and MC-LR were virtually absent in the phytoplankton from Coal Lake in all 3 years (Figs. 1*d*, 2*d*, and 3*d*). *Microcystis aeruginosa* was absent from Narrow Lake on three of four sampling dates; on 20 September there were a few cells (1221 cells·mL^{-1}). MC-LR was not detected in phytoplankton samples from Narrow Lake. Seasonal changes in MC-LR concentration in the phytoplankton from all lakes tracked changes in *M. aeruginosa* abundance closely: the two were highly correlated ($r = 0.67$, $P < 0.0001$, df = 121).

Microcystin in lake water

Aqueous microcystin concentration in lake water (expressed as MC-LR equivalents) was highest in the two hypereutrophic lakes. In Driedmeat Lake, aqueous microcystin

Fig. 3. Seasonal changes in surface water temperature and chlorophyll *a* (*a–c*), *Microcystis aeruginosa* abundance and MC-LR concentration in the phytoplankton (*d–f*). MC-LR concentration in three gastropod species (*g–i*) for Coal (left panels), Driedmeat (middle panels), and Little Beaver (right panels) lakes for 1994. Except for gastropods, samples were collected from the 0- to 2-m stratum of the water column in Coal and Driedmeat lakes, and from the 0- to 1-m stratum in Little Beaver Lake. Asterisks in Figs. 3*g*–3*i* indicate nondetectable MC-LR (<1 µg·g^{-1} MC-LR) in all three gastropod species. MC-LR was quantified by HPLC.

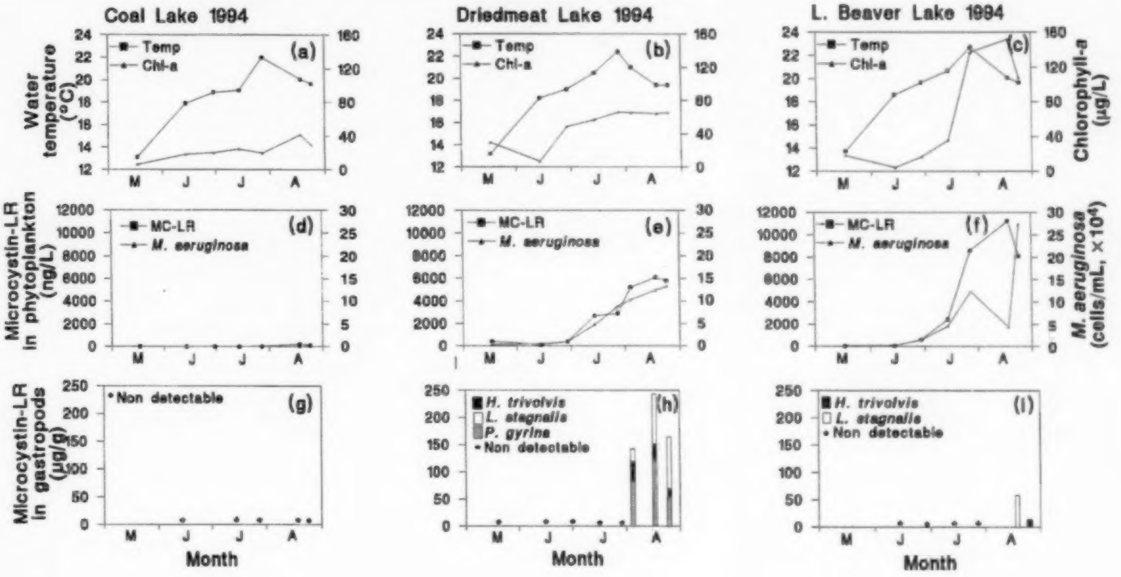
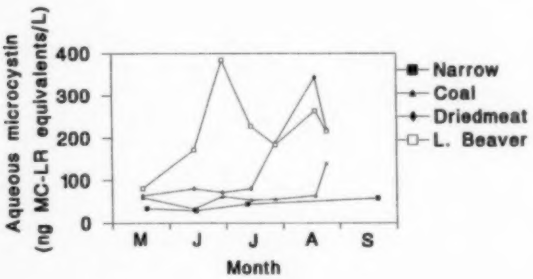


Fig. 4. Seasonal changes in aqueous concentration of total microcystin in the upper 2 m of the water column (Coal, Driedmeat, and Narrow lakes) or the upper 1 m of the water column (Little Beaver Lake) during 1994. Total microcystin concentration was determined by protein phosphatase assay and is expressed as MC-LR equivalent units per litre.



increased over the summer, peaking at 344 ng·L^{-1} in mid-August (Fig. 4). The trend of increasing microcystin concentration in the water paralleled the trend of increasing MC-LR in the phytoplankton in Driedmeat Lake ($r = 0.89$, $P = 0.006$, df = 5; Figs. 4 and 3*e*). In contrast, microcystin concentration in the water from Little Beaver Lake in 1994 peaked on 29 June (284 ng·L^{-1}) when MC-LR concentration in the phytoplankton was low (Figs. 4 and 3*f*). Aqueous microcystin concentration was close to our detection limit in Narrow and Coal lakes, generally below 60 ng·L^{-1} (Fig. 4). Aqueous microcystin concentrations for all lakes combined were correlated to MC-LR in the phytoplankton ($r = 0.83$, $P < 0.001$, df = 24).

Zooplankton

MC-LR was detected by HPLC in 27 of the 68 zooplankton samples; all collected from Driedmeat and Little Beaver lakes (the lakes containing the highest MC-LR concentrations in phytoplankton and water). MC-LR concentrations in the zooplankton communities peaked in August in Driedmeat Lake (Figs. 1*h* and 2*h*) but was highest from mid-May to late August in the Little Beaver Lake (Figs. 1*i* and 2*i*). The peaks in MC-LR concentration in the zooplankton communities coincided with peak MC-LR in phytoplankton ($r = 0.69$, $P < 0.001$, df = 66), although there were occasions where peak MC-LR in zooplankton did not correspond to maximum MC-LR concentration in the phytoplankton (Figs. 2*e* and 2*h*).

Substantive seasonal shifts in zooplankton community composition occurred in the lakes. In Little Beaver Lake, these shifts occurred following periods when MC-LR concentration was at its highest in the zooplankton community. MC-LR concentration decreased in the zooplankton during these shifts. For example, up to a 17-fold decrease in MC-LR concentration in the zooplankton community (Fig. 1*j*) coincided with a shift from *Daphnia pulex* (in June and July 1992) to *Diaptomus oregonensis* and *Diaphanisoma leuchtenbergianom* in July and August 1992 (Fig. 1*l*). In contrast, similar shifts in zooplankton community structure were not observed in Driedmeat Lake in 1992 and 1993 (Figs. 1*k* and 2*k*, respectively). In addition, no single zooplankton species was dominant during periods when MC-LR concentration in the zooplankton community peaked.

For the majority of zooplankton samples (i.e., 61 of 68 samples) there was agreement between total microcystin and MC-LR concentrations determined by PP1c assay and HPLC, respectively . These results are consistent with observations that the major microcystin analogues produced by phytoplankton in Alberta lakes is MC-LR (Kotak et al. 1993; Boland et al. 1993; Craig et al. 1993). However, in a small number of the samples, other microcystins may have been present.

Macroinvertebrates

MC-LR was detected in three species of gastropods (*Lymnaea stagnalis*, *Helisoma trivolvis*, and *Physa gyrina*) collected from Driedmeat and Little Beaver lakes, but only in August (Figs. 3*h* and 3*i*), when MC-LR in the phytoplankton was at its highest (Figs. 3*e* and 3*f*). MC-LR was not detected in gastropods collected from Coal and Narrow lakes or in any other macroinvertebrates. On 3 and 17 August in Driedmeat Lake, *P. gyrina* had the highest MC-LR concentration (90 and 121 $\mu g \cdot g^{-1}$, respectively), while on 23 August, *L. stagnalis* had the highest concentration (96 $\mu g \cdot g^{-1}$). In Little Beaver Lake, MC-LR was detected only once in *L. stagnalis* (on 17 August; 54 $\mu g \cdot g^{-1}$) and *H. trivolvis* (on 23 August; 11 $\mu g \cdot g^{-1}$). However, no samples of *H. trivolvis* and *P. gyrina* on 17 August and *L. stagnalis* and *P. gyrina* on 23 August were collected. The nondetectable values, indicated by an asterisk in Figs. 3*h* and 3*i*, represent nondetectable MC-LR for all three gastropod species. Detectable MC-LR concentrations in the gastropods from Driedmeat Lake also coincided with high aqueous total microcystin in the lake water (Fig. 4). However, the same was not true in Little Beaver Lake, where MC-LR was nondetectable in gastropods even when total dissolved microcystin concentration was highest (from late June onward). Therefore, MC-LR detected in gastropods likely originated from the phytoplankton, not from toxin taken up from an aqueous source.

Fish

Northern pike and white sucker were collected from Driedmeat Lake after MC-LR had been at its highest in the phytoplankton for almost 1 month (mean 2849 $ng \cdot L^{-1}$ from 4 to 30 August; Fig. 2*e*). Eight livers from the northern pike and 23 from white sucker were extracted and analyzed for MC-LR by HPLC. MC-LR was not detected in any of the livers from the two species of fish, despite stomach contents that suggested that the fish should be exposed to the toxin through an oral route. For example, the stomachs of the northern pike contained gastropods (which were collected from Driedmeat Lake on the same days as the fish and contained 58 $\mu g \cdot g^{-1}$ MC-LR equivalents by PP1c assay) as did the stomachs of the white sucker, in addition to unidentified phytoplankton.

HPLC-linked PP1c bioassay

To confirm the presence of MC-LR and possibly other microcystins in fish liver, phytoplankton, and invertebrate samples, HPLC-linked PP1c bioassay was used (Figs. 5 and 6). MC-LR was not detected in the fish livers by HPLC or HPLC-linked PP1c assay. In the phytoplankton, zooplankton, and gastropod samples, the main peak in the HPLC chromatogram (and the one with the highest PP1c inhibition) always corresponded to the retention time of MC-LR (Figs. 5 and 6). For example, the HPLC chromatogram for the Driedmeat Lake phytoplankton sample contained one major peak with several smaller peaks (Fig. 5*a*). The largest peak in the chromatogram, with a retention time of 8.60 min, corresponded to the retention time of the MC-LR standard (Fig. 5*b*). Four HPLC fractions inhibited PP1c (Fig. 5*a*). The fraction with the greatest inhibition corresponded to the MC-LR peak. In addition, another peak, with a retention time of 9.07 min also inhibited PP1c. Two other peaks, both preceding MC-LR, had much lower PP1c inhibition (Fig. 5*a*). Therefore, the major microcystin in the phytoplankton was likely MC-LR, although another peak (likely another microcystin) also inhibited PP1c. We have previously shown by fast atom bombardment – mass spectrometry, that the peak in phytoplankton samples corresponding to the retention time of MC-LR standard, has the same molecular mass as that of MC-LR (Kotak et al. 1993).

To further confirm the presence of MC-LR in the zooplankton and gastropod samples, each sample was spiked with MC-LR and rerun on the HPLC (Fig. 6). The peak area of the presumed MC-LR peak in the zooplankton and gastropod samples increased by 1.78- and 1.59-fold, respectively, a recovery of 98.7 and 99.6%, respectively, of the added MC-LR standard. This suggested that the original peak in question in the two samples was MC-LR.

Discussion

Few studies have examined whether cyanobacterial toxins can accumulate in aquatic food webs, at what concentrations they are present in the various compartments, and how the trophic status of a water body may influence toxin concentrations. In companion studies, we have shown that microcystin-LR (MC-LR) concentration in phytoplankton can be highly dynamic, reflecting, at least partially, changes in the abundance or biomass of *M. aeruginosa* within a lake, temporally within and between years, and even over 24 h (Kotak et al. 1993, 1995). Therefore, the occurrence of MC-LR in food web compartments will depend on the occurrence and biomass of *M. aeruginosa* in the phytoplankton. Our data indicate that lakes of low productivity (such as oligo-mesotrophic Narrow Lake) and even some hypereutrophic lakes (such as Coal Lake) may have a low potential for supporting microcystin-producing phytoplankton. Kotak (1995) has shown that consistently low MC-LR concentrations in Coal Lake phytoplankton may be related to seasonal total nitrogen to total phosphorus (TN:TP) ratios that exceed 5:1, whereas higher MC-LR concentrations in Driedmeat and Little Beaver lakes correspond to TN:TP ratios that are often less than 5:1. Trophic status, therefore, likely will influence the occurrence and concentration of MC-LR in aquatic food webs by restricting the distribution of *M. aeruginosa*.

Aqueous microcystin concentration and MC-LR concentration in the phytoplankton were both highest in Driedmeat and Little Beaver lakes. Microcystins are considered endotoxins because the majority of the toxin is found within the cells. When the cell wall of the cyanobacterium is compromised, as a result of chemical treatment (Kenefick et al 1993; Lam and Prepas 1995; Lam et al. 1995*a*, 1995*b*) or natural senescence (Berg et al. 1987), microcystins are released into the water. Despite the much higher MC-LR concentrations in the

Fig. 5. HPLC chromatogram of phytoplankton collected from Driedmeat Lake on 17 August 1994 (*a*) and MC-LR standard (*b*). Analysis was on a Waters LC I HPLC with UV detection at 238 nm. The sample and standard were run under linear gradient conditions (20–30% acetonitrile in 10 mM ammonium acetate in 10 min, then 20% from 10 min to the end of the run (30 min)). Boxes in (*a*) indicate fractions collected for protein phosphatase inhibition. Values above the boxes indicate microcystin concentration of the fraction, as quantified by protein phosphatase assay, and are expressed as nanograms MC-LR equivalents per millilitre. Values <1 ng·mL^{-1} are considered nondetectable.

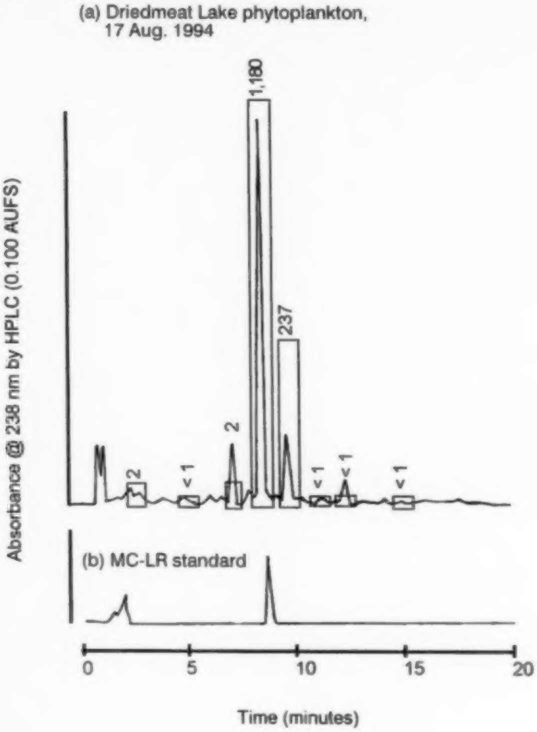
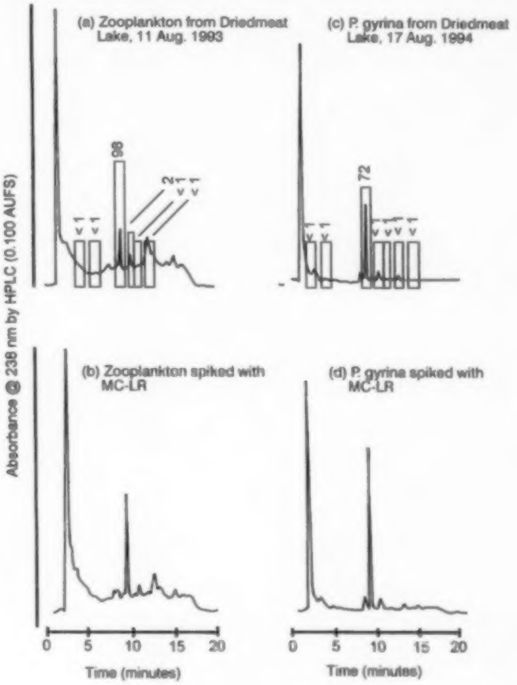


Fig. 6. HPLC chromatogram of zooplankton collected from Driedmeat Lake on 11 August 1993 (*a*), the above zooplankton sample spiked with MC-LR standard (*b*), *Physa gyrina* (gastropod) sample collected from Driedmeat Lake on 17 August 1994 (*c*), and the above gastropod sample spiked with MC-LR (*d*). Analysis was on a Waters LC I HPLC with UV detection at 238 nm. The sample and standard were run under linear gradient conditions (20–30% acetonitrile in 10 mM ammonium acetate in 10 min, then 20% from 10 min to the end of the run (30 min)). Boxes in Figs. 6*a* and 6*c* indicate fractions collected for protein phosphatase inhibition. Values above the boxes indicate microcystin concentration of the fraction, as quantified by protein phosphatase assay, and are expressed as nanograms MC-LR equivalents per millilitre. Values <1 ng·mL^{-1} are considered nondetectable.



phytoplankton in Little Beaver than in Driedmeat Lake, concentrations of aqueous microcystin in the two lakes were similar. Degradation by microbial action (Lam et al. 1995*a*) and (or) photolysis (Tsuji et al. 1995), as well as dilution, may account for the similar concentrations of aqueous microcystin in these two lakes.

Aquatic invertebrates analyzed for MC-LR by HPLC covered a diversity in trophic feeding levels, from detritivores (such as *Gammarus lacustris* and *Chironomus* spp.), herbivores (zooplankton such as *Daphnia pulex*, *Bosmina longirostris*, and *Diaphanisoma leuchtenbergianom* and gastropods such as *Physa gyrina*, *Lymnaea stagnalis*, and *Helisoma trivolvis*) to predators (zooplankton such as *Acanthocyclops vernalis* and the predacious water beetle, *Dytiscus* sp.). However, MC-LR was only detected in zooplankton communities and gastropods (i.e., in the grazers). In general, the highest MC-LR concentrations in the zooplankton and gastropods corresponded fairly well to high concentrations in the phytoplankton although there were exceptions. The correlation between MC-LR concentration in the zooplankton and phytoplankton (r = 0.69) suggests that MC-LR may be present in the zooplankton as result of filter feeding on the toxin-containing *M. aeruginosa* cells. If so, there should be a relationship between the abundance of herbivorous grazers and MC-LR in the zooplankton. MC-LR concentration in the zooplankton was correlated to the relative abundance of all the grazers combined (*D. pulex*, *B. longirostris*, *D. leuchtenbergianom*, and *D. oregonensis*, $r = 0.35$, $P < 0.05$, df = 36) but not to individual species ($P > 0.09$).

It is possible that accumulation of MC-LR in zooplankton may also occur by uptake for the toxin in aqueous form, directly from the water. Unfortunately, this hypothesis cannot be addressed in our study as water and zooplankton samples for MC-LR were not collected in the same years. However, the absence of detectable MC-LR in macroinvertebrates (e.g., damselfly and dragonfly larvae, chironomids) collected in 1994 from Driedmeat and Little Beaver lakes (the lakes with the highest aqueous microcystin concentrations), suggests that either MC-LR is not taken up from the water, or that given its high water solubility, the toxin is rapidly eliminated. DeMott et al. (1991) found that purified, aqueous MC-LR was lethal to *Daphnia pulicaria*, *D. hyalina*, *D. pulex*, and *Diaptomus birgei* in laboratory experiments, and thus, MC-LR must be taken up from the water. However, concentrations of MC-LR used in their trials were unrealistically high (500–50 000 $\mu g \cdot L^{-}$) compared with the total aqueous microcystin concentrations reported in our study lakes (maximum concentration of 0.34 $\mu g \cdot L^{-1}$). Given this evidence, we believe that the MC-LR detected in the zooplankton and gastropods from the lakes in the present study was toxin that was present in the ingested *M. aeruginosa* cells.

Only one other study has quantified microcystin concentrations in freshwater zooplankton communities in situ. Watanabe et al. (1992) detected concentrations of microcystins -LR, -YR, and -RR from 75 to 1387 $\mu g \cdot g^{-1}$ of the three analogues in zooplankton samples collected from Lake Kasumiguara, Japan. These concentrations are up to 9 times higher than the highest total microcystin concentration detected by PP1c and up to 20 times higher than the highest MC-LR concentration detected by HPLC from zooplankton collected during the present study. Watanabe et al. (1992) suggested that *Bosmina fatalis* was the principal zooplankton species responsible for accumulation of the three microcystin variants, although they did not attempt to quantify the relationship between microcystin concentrations and zooplankton species composition.

It is not known whether the concentrations of MC-LR detected in the zooplankton from the two lakes were sufficient to cause mortality in the various zooplankton species and, hence, cause changes in zooplankton community composition. Given the results of DeMott et al. (1991), it is unlikely that the aqueous microcystin concentrations in Driedmeat and Little Beaver lakes would cause mortality in any of the zooplankton species found. In addition, high MC-LR concentrations in the zooplankton in Driedmeat Lake during late August in 1992 (Fig. 1*h*) and during late May and throughout August in 1993 (Fig. 2*h*) were not proceeded by major shifts in zooplankton species composition (Figs. 1*k* and 2*k*, respectively). Therefore, it did not appear that the high MC-LR burdens resulted in a change in zooplankton species composition in Driedmeat Lake. However, large shifts in zooplankton species composition did occur in Little Beaver Lake in 1992 and 1993 following peak MC-LR concentrations in the zooplankton (Fig. 1*i*, 1*l*, 2*i*, and 2*l*). The relative importance of MC-LR in affecting zooplankton community composition, however, is unknown. It has been suggested that cyanobacterial toxins are produced as a defense mechanism against grazing pressure exerted by herbivorous zooplankton (Lampert 1981; DeMott and Moxter 1991) and, therefore, may control zooplankton competitive relations and species composition (Fulton and Paerl 1988). In response, the zooplankton have in turn evolved mechanisms to reduce their susceptibility to the toxins. These mechanisms include increased physiological resistance to the toxins themselves (DeMott et al. 1991), reduction in filter feeding activity (Lampert 1981, 1982), and rejection of toxic forms over nontoxic forms after the cells have been captured (DeMott and Moxter 1991). Any one of these mechanisms could help explain the variable concentrations of MC-LR detected in the zooplankton from Driedmeat and Little Beaver lakes and changes in zooplankton species composition.

MC-LR that was detected in three species of gastropods collected from Driedmeat and Little Beaver lakes in 1994 (Figs. 3h i) was also likely a result of grazing activities. The changes in MC-LR concentration in *Lymnaea stagnalis* and *Physa gyrina* roughly followed the changes in MC-LR concentration in the phytoplankton. In contrast, MC-LR concentration in *Helisoma trivolvis* was highest on the first sampling date in August and declined thereafter (Fig. 3*h*). On the last sampling date, 23 August, many of the *H. trivolvis* were either dead or appeared unhealthy. It is unknown whether the high MC-LR concentrations in the phytoplankton were responsible for the large number of dead *H. trivolvis* on this date. Gastropods in general are not considered filter feeders (Calow 1970; Elder and Collins 1991), preferring to graze on periphyton (attached algae) which adhere to the surface of macrophytes. The source of the MC-LR in the gastropods in the present study is likely from *M. aeruginosa* cells and colonies that settled out of the water column and became trapped in the abundant macrophytes in Driedmeat and Little Beaver lakes. The gastropods were almost always associated with dense growths of *Potamogeton pectinatis*, *P. richardsonii*, and *Myriophyllum spicatum*, which had dense films of *M. aeruginosa* on their surfaces.

Only one study has examined microcystin accumulation in macroinvertebrates. Erikkson et al. (1989) examined the accumulation of a microcystin analogue (detected by HPLC) by the freshwater mussel, *Anodonta cygnea*, exposed to a hepatotoxic laboratory culture of the cyanobacterium *Oscillatoria agardhii*. Although the mussels accumulated high concentrations of the microcystin in the tissue (70 $\mu g \cdot g^{-1}$ after a 15-day exposure), they were also exposed to high concentrations of the microcystin in pure culture (2000–3000 μg toxin·g^{-1} or 40–60 μg cellular toxin·L^{-1}). Gastropods from our field study were exposed to much lower concentrations of MC-LR. The highest concentration of MC-LR in the phytoplankton during our study was 11.2 $\mu g \cdot L^{-1}$. Therefore, it may be difficult to extrapolate the results of Eriksson et al. (1989) to natural lake settings.

Accumulation of other protein phosphatase inhibitors have also been reported in marine and brackish-water environments. Nodularin, a cyanobacterial hepatotoxin, was detected in blue mussels (*Mytilus edulis*) during a hepatotoxic bloom the cyanobacterium *Nodularia spumigena* in Peel–Harvey estuary, Western Australia (Falconer et al. 1992). Gut contents of the mussels contained filaments of *Nodularia spumigena*, and the highest toxicity (detected by mouse bioassay) was associated with the gastrointestinal tract of the mussels. No toxicity was observed for non-intestinal tissues. Additionally, motuporin (a derivative of nodularin) was isolated from the marine sponge *Theonella swinhoei* (deSilva et al. 1992), diarrhetic shellfish poisons okadaic acid and dinophysitoxin-1 from marine shellfish (Holmes 1991) and MC-LR from crab larvae, copepods, amphipods, mussels, and pen-reared Atlantic salmon afflicted with netpen liver disease (Andersen et al. 1993; Chen et al. 1993).

MC-LR was not detected in the livers of fish collected from Driedmeat Lake, despite the fish potentially being exposed for more than 1 month in 1993 to high concentrations of MC-LR in the phytoplankton, aqueous microcystin, and MC-LR in food items (e.g., gastropods). Most of the microcystins in the fish livers were likely covalently bound to protein phosphatases (Toivola et al. 1994) and, therefore, would not be detected by our current HPLC method. The absence of detectable microcystin in the liver (or in the flesh or any other organ; data not shown) does not imply that the fish were not exposed to the toxins or that the toxin had no health effect on the fish. Binding of any microcystin to protein phosphatases in the liver is a pathological result in itself. This would likely be manifested as focal, multifocal, or massive zones of coagulative or liquifactive hepatocyte necrosis, as is seen in rainbow trout that have been exposed to MC-LR by intraperitoneal injection (Phillips et al. 1985; Kotak et al. 1996). Although long-term (i.e., >96 h) exposure of fish to microcystins has not been studied, we would hypothesize that chronic exposure of natural fish populations to microcystins may lead to severe necrosis of hepatocytes, loss of liver function, and perhaps liver tumour promotion or mortality. Such physiological stress would clearly have an impact on growth rates in the fish.

There has been one study published that has confirmed that microcystins can have a substantive and, at times, severe impact on commercially important fish populations. Kent et al. (1988) and Kent (1990) reported severe liver lesions, which they termed netpen liver disease (NLD), and mortality in netpen-reared Atlantic salmon since 1986. The liver lesions (Kent et al. 1988; Kent 1990) were identical to those caused by intraperitoneal exposure of the salmon to MC-LR in laboratory experiments (Andersen et al. 1993). The disease in fish held in netpens was not related to diet, could not be experimentally transmitted (therefore, was not due to viral or bacterial action), was not related to anthropogenic contamination and became progressively worse over the summer in each year. Subsequently, Andersen et al. (1993) successfully isolated a compound that was chromatographically indistinguishable from MC-LR from the livers of salmon inflicted with NLD and from biota fouling the netpens, utilizing HPLC-linked PP1c bioassay. MC-LR was not detected in the livers of fish not afflicted with NLD. The more sophisticated extraction and cleanup steps used in the isolation and purification of MC-LR from the salmon livers (including semi-preparative HPLC followed by the use of a microbore analytical column) compared with our method of sample extraction and cleanup and the much greater mass of liver tissue extracted (22.7 g dry weight versus 2–3 g in the present study) may explain why we did not detect MC-LR in the livers of northern pike and white sucker. Andersen et al. (1993) is the first study to document a direct (and severe) effect of MC-LR on fish populations in the field. They suggested that the fish were exposed to the MC-LR via feeding on organisms fouling the netpens.

In summary, the potential for MC-LR to occur in organisms of aquatic food webs in our study lakes was related to the trophic status of the water body, strongly influenced by the occurrence and abundance of the toxin producer (*M. aeruginosa* in this case). MC-LR, accumulated in herbivorous invertebrates, could potentially affect the health of organisms at higher trophic feeding levels (e.g., fish). Although not studied to date, there is also a strong possibility of transfer of MC-LR from aquatic food webs to terrestrial food webs, by waterfowl and shorebirds, for example, feeding on contaminated gastropods and zooplankton. Accidental ingestion of cyanobacterial hepatotoxins in drinking water has repeatedly caused poisonings and death in animals worldwide (Schwimmer and Schwimmer 1968; Edler et al. 1985). Given this scenario, more attention needs to be directed to the ecology of toxin-producing cyanobacteria and the role of the toxins in food webs.

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Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua*

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Abstract: We analyzed the maternal and seasonal contributions to variation in egg sizes and spawning characteristics among captive Atlantic cod, *Gadus morhua*, from the Bay of Fundy, Canada. Spawning occurred from late December to mid-June. No seasonal change in egg sizes was apparent among the eggs pooled from all females. At the female level, those that began spawning later in the year spawned for a shorter duration and had fewer batches, but these batches were larger and released at higher frequencies. Effects due to female ($n_f = 10$) and batches-within-female ($n_{b,f} = 73$) accounted for 35 and 26% of the total variance in egg diameters, respectively. Sizes of eggs from batches early in the spawning season were predictive of sizes in later batches from the same female. Female size was not related to egg size, but females of higher condition had larger eggs. The batch effect was largely due to seasonal declines in egg sizes of the separate females and was related to female condition. Despite the use of constant temperatures in this study, available information implicates temperature as a dominant factor contributing to seasonal changes in egg sizes in nature.

Résumé : Nous avons étudié l'influence maternelle et celle de la saison de ponte sur les variations de grosseur et des caractéristiques génésiques des oeufs pondus par des femelles de la morue franche, *Gadus morhua*, qui provenaient de la baie de Fundy, Canada. La ponte s'est échelonnée de la fin décembre à la mi-juin. Nous n'avons observé aucune variation saisonnière dans la taille des oeufs provenant de toutes les femelles et regroupés. Quant aux femelles, celles qui commençaient plus tard dans l'année pondaient moins longtemps et produisaient moins de masses d'oeufs, mais qui étaient plus importantes et pondues à une fréquence accrue. Les effets attribuables aux femelles ($n_f = 10$) et le nombre de masses d'oeufs par femelle (n_{mf} = 73) permettaient d'expliquer 35 et 26 %, respectivement, de la variation totale du diamètre des oeufs. La taille des oeufs pondus tôt en saison était indicatrice de celle des oeufs que les mêmes femelles pondraient par la suite. Il n'existe pas de corrélation entre la taille des femelles et la grosseur des oeufs, cependant les femelles en meilleur état pondaient de plus gros oeufs. L'effet attribuable aux masses d'oeufs était largement associé à la diminution saisonnière de la taille des oeufs de femelles considérées isloément et il était en rapport avec l'état des femelles. Nous n'avons pas fait varier la température de l'eau au cours de cette étude, mais les travaux antérieurs nous ont appris qu'elle constitue une cause dominante des variations saisonnières de la grosseur des oeufs en milieu naturel.
[Traduit par la Rédaction]

Introduction

The sizes of egg within stocks of Atlantic cod, *Gadus morhua*, have long been known to vary (Earll 1880; Williamson 1895). Early ichthyoplankton collections revealed that eggs of Atlantic cod decrease in size as the spawning season advances (Heinke and Ehrenbaum 1900; Ehrenbaum 1905, 1909; Kramp 1913), and some type of maternal effect has often been invoked to account for these seasonal changes. That females differ in the sizes of their eggs has since been confirmed from measurement taken on ripe, field-collected Atlantic cod (Grauman 1964; Solemdal 1970; Knutsen and Tilseth 1985). The suggestion that smaller or younger females have smaller eggs dates at least to Earll (1880). In addition, Sund et al. (1938), among others, reported that older females arrive on the spawning grounds earlier than their younger counterparts. The combination of these two age-related reproductive patterns is one of several explanations for the seasonal decline in egg sizes of Atlantic cod (hereafter cod with occasional identifiers for separate stocks).

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Variation in the sizes of marine fish eggs has been associated with numerous factors besides female status and season of spawning, including population origin, latitude, temperature, salinity, and egg batch sequence, among others (Chambers 1997). Although cod is a well-studied teleost, the effects of these factors on egg size, acting singly or in combination, remain largely unassessed. Cod is a particularly good candidate for the analysis of environmental and maternal effects on egg size variability because it is broadly distributed, exhibits a wide range of sizes and ages of reproductive females (reproductive lifespan >20 years under unexploited conditions), and individual females spawn multiple batches of eggs over several to many months of the year. Although these effects have not been quantified in earlier studies of egg size variability, it appears that they vary from year to year. For example, over a 5-year sampling period, the decline in egg diameters during the spawning season of Northeast Arctic cod ranged from 2 to 7% (Wiborg 1960). A year effect in the seasonal changes in egg sizes has also been reported for cod from the Scotian Shelf, Canada (Miller et al. 1995).

Cod spawn spontaneously in captivity even when mated pairs of adults are maintained in isolation. This behaviour has been used to advantage in studies of the spawning characteristics of individual female cod within and across years (Solemdal 1970; Howell 1984; Kjesbu 1989, 1994; Kjesbu et al. 1991, 1992). To date, however, studies of broodstock cod have been largely descriptive. The set of relationships between female attributes, spawning duration and intensity, and egg size has not apparently been subjected to variance analyses.

This study is based on correlative and variance analyses of eggs obtained from individual captive cod over an entire season of spawning. Our primary purpose is to analyze the variability in sizes of eggs of cod and to assess the maternal and seasonal contributions to this variability. We also, however, consider the numbers of eggs shed and the timing of their release. We begin by inspecting the overall pattern of reproductive output, including the seasonal patterns of egg sizes, abundances of eggs released, and batch frequencies, but without regard to female or batch origin. Next, we evaluate these features at the level of the female and estimate the variability in egg size attributable to females and batches-within-females. Following this, we estimate the strengths of relationships between the status of a female and the size, number, and timing of release of her eggs. Lastly, we assess the seasonal changes in egg sizes within females.

The variation in reproductive features described for a single group of captive cod cannot be unconditionally applied to other situations or stocks, let alone other species. Nonetheless, we believe an appraisal of the patterns and sources of variation in reproductive features of cod can only enhance life-historical and population analyses of this important member of the North Atlantic ichthyofauna. We view egg size as a key feature in the early life of cod and marine fishes in general and document elsewhere that it is predictive of the size and energy reserves of young larvae (Chambers et al. 1989; Chambers 1993, 1997; see also Miller et al. 1995).

Methods

Broodstock maintenance and data collection

Cod were collected as juveniles from 1980 to 1981 by otter trawl from Passamaquoddy Bay, New Brunswick, Canada (45°05′N, 67°00′W), and held in 2 m diameter flow-through indoor tanks at the Biological Station, Department of Fisheries and Oceans, in St. Andrews, N.B. The tanks were maintained at ambient photoperiod, temperature (range 0–12°C), and salinity (28.5–31 ppt). The cod were offered fresh or frozen fish or squid three times per week.

In November 1983, each of 10 mature male–female pairs was placed in its own 1 m diameter flow-through tank, which was supplied with filtered seawater. Temperatures were maintained at 4 ± 0.2°C (mean ± nominal range) throughout the experimental period. Cod were offered fresh or frozen shrimp or herring thrice weekly, but feeding by the spawning fish was variable, generally discontinuing during intensive spawning. Female total length (TL; centimetres) and mass (grams wet weight) were measured near the beginning and end of the spawning season (January 4 and June 5, respectively). Growth in length and mass during the spawning season and condition before and after spawning (Fulton's $k = 100(\text{wet weight})\ \text{TL}^3)$ were calculated from these measurements.

A collector capable of retaining floating and sinking eggs was placed on the effluent line of each tank. These collectors were checked each morning from December 1983 to July 1984, and the collected eggs were preserved in a 4% seawater-buffered formalin. The volume of the collected eggs never exceeded 30% of the container volume.

All of our analyses of egg numbers and sizes are based on the eggs shed by females and retained in the collectors. We define a batch of eggs as the eggs collected over an interval that is preceded and followed by a period of ≥48 h during which few or no eggs were collected and assume these eggs resulted from one spawning event. Given the relatively fast turnover rates of water in the tanks (≈10 min), eggs from most batches were collected in 1 day, although eggs from some batches were collected over a period of several days. When this occurred, one day's collection was generally much larger than the others, i.e., the modal day. Eggs collected on the modal day ranged from 35 to 95% of the total collected for a batch. Two-thirds of the 79 batches collected from the 10 pairs of adults were comprised of eggs released on 1 day. The remaining third included eggs collected over a period of 2–3 days.

The number of eggs released and collected per mated pair per day was estimated volumetrically after counting the number of eggs per unit volume in a subsample of the eggs collected from a tank. We interpret our estimates of various measures of fecundity as "realized fecundities" as they do not include ovarian eggs, some of which may be resorbed by the female (Kjesbu et al. 1991). We estimated a female's batch fecundity as the number of eggs collected during a spawning event. We computed seasonal fecundity per female as the sum of all respective batch fecundities. We define relative seasonal fecundity for a female as the number of eggs collected for the entire season per unit weight of female (number per gram wet weight). The end-of-season female weight was used as the divisor in the calculation of relative fecundity as it more closely approximates an ovary-free weight.

We quantified egg size by the external diameter of preserved eggs. Measurements were taken from samples from every batch of eggs released from each female. If eggs were shed over several consecutive days, the modal day was used as the sample source. Sampling was achieved by thoroughly agitating the eggs in their preservation container and collecting two 5-mL aliquots with a glass tube. Each aliquot was placed on a numbered grid and a subsample of 30–40 eggs was chosen at random for measurement. We projected a magnified image of these eggs onto a digitizing tablet and registered the outside diameter of each egg with a digitizing stylus. Resolution of the measurement for an individual egg exceeded 0.01 mm. The accuracy of this technique was verified by comparing the estimates of egg diameters with those obtained by direct measurement using an ocular micrometer at 25× magnification and by digital image analysis of videotapes of the eggs recorded at 50× magnification. We did not attempt to correct for egg shrinkage due to preservation because the magnitude of formalin-induced shrinkage of cod eggs is slight, being <2% of diameter of fresh eggs (Hislop and Bell 1987).

Most of the eggs inspected were fertilized, but this could not be documented in every case because of the large number of eggs collected (≈4.8 million), and the period between spawning and preservation was often too short to permit noticeable embryonic development, e.g., first cleavage occurs 5–6 h after fertilization at 5°C (Kjørsvik and Lønning 1983). We assume that the measured eggs had reached their full post-fertilization diameters, which are

≈3% greater than their diameters at fertilization. This assumption is reasonable because the full expansion of the perivitelline space is achieved within 1 h after fertilization (Kjørsvik and Lønning 1983). Cod are reported to spawn predominantly at night (Brawn 1961; Ferraro 1980), and the eggs from the collectors were not preserved before 08:00.

Data processing and statistical analyses

Our primary goal was the analysis of the magnitude, pattern, and sources of size variability of eggs that are likely to produce viable larvae. We could not take the eggs through to hatching to verify viability so we chose instead to use egg size as an indicator of viability. This is a coarse criterion, but we used it to eliminate eggs of obviously abnormal sizes, which are likely to be infertile. Unusually large, irregularly shaped ova are often high in water content and these characteristics are generally indicative of overripening (Kjørsvik et al. 1990). Such eggs were more common late in a female's spawning period although some level of abnormality occurred in earlier batches of eggs. Prior to our analysis, we therefore inspected the initial size–frequency distributions of eggs using a three-tiered process of detecting outliers.

The first tier was at the within-batch level and was designed to detect statistical outliers in each of the original 79 batches of eggs. Following Wilkinson (1992), we considered an egg to be an outlier if its diameter was more extreme than a value 1.5× the interquartile range added to the upper quartile or subtracted from the lower one. This procedure identified a total of 160 eggs of unusual size of which 143 were excessively large. These eggs were distributed among all females (range: 6–33 outliers per female).

The second tier was at the level of batches-within-females and was designed to identify entire egg batches from a female that were of unusual size. The same outlier criterion was used but this time applied to the mean egg diameters per batch for all batches from a female. This procedure eliminated one batch of excessively large eggs from female No. 6 (the numbering scheme for females used throughout is an ascending ranking of the 10 females based on their average egg sizes).

The third tier of filtering was also at the level of batches-within-females and used mean egg diameters, but it was designed to detect batches of abnormally sized eggs shed late in the period of spawning. We expected the eggs from a female to decrease in size during the spawning period based on previous studies of cod and other gadids (e.g., Hislop et al. 1978; Kjsebu 1989; Hinckley 1990). We further anticipated that the last batch released by a female could comprise a small number of residual eggs, abnormally large for that time in the spawning season (personal observation). A check for batch outliers based on mean values, as in the second tier of filtering, would not identify an unusual upward shift in egg sizes of the terminal batch if its mean did not exceed the previously described outlier criterion. Therefore, in the third tier of filtering, we used the magnitude of statistical influence of batch mean egg diameters to the linear seasonal trend in mean egg sizes (Madansky 1988). This procedure eliminated five batches of unusually large, late-season eggs. Four of these were the terminal batches of eggs from four females (Nos. 1, 3, 8, 10), and the fifth was the penultimate batch from female No. 10.

Overall, the occurrence and numbers of outliers per female were not related to any female feature or spawning characteristic. The remaining 2054 eggs from 73 batches produced by 10 females were subjected to statistical analysis.

We conducted four analyses on these data. First, we considered the pooled data without regard to female and batch identity. This provided estimates of the initiation and duration of the spawning season, spawning intensity, overall characteristics of the egg size distribution, and seasonal trends in the numbers and sizes of eggs.

Second, and of primary interest in our study, we partitioned the observed variance in egg sizes into female, batches-within-female, and within-batch (residual) components. This was achieved by employing a two-level random-effects analysis of variance (ANOVA) with batch number nested within female. We quantified then standardized the components of variance due to females and batches. Variance components for each term were solved algebraically after equating the observed mean squares to their expected values. These were converted to percentage contributions to the total variance in egg sizes. We also partitioned the egg size variance for each female into fractions attributable to batch and within-batch components.

Differences in the number of batches analyzed per female (4–11), and the number of eggs analyzed per batch (22–36) led to an unbalanced ANOVA design. This precluded calculation of an exact *F* value for testing the hypothesis of no differences in egg size variances among females. Therefore, an approximate *F* was constructed by synthesizing the among-batch mean squares using the method of Satterthwaite (Sokal and Rohlf 1981, p. 298).

Prior to conducting the ANOVA, the distributions of egg diameters were inspected for their agreement with the ANOVA assumptions of normality within and homoscedasticity among batches of eggs. The distributions were non-normal and heteroscedastic (Levene's test; Madansky 1988). To mitigate the violations of assumptions, we log transformed the original observations of egg diameters (ED) with the expression, $\log_e(10 \times \mathrm{ED})$. Tests of hypotheses about group means (e.g., no female effect, no batch effect) are robust to violations of ANOVA assumptions, and the design was only moderately unbalanced (max n/min $n = 1.6$). However, estimates of variances and their confidence limits are not robust with respect to unbalanced random-effects ANOVAs with heteroscedastic errors (Scheffé 1959). We, therefore, interpret our calculated variance components as approximations.

In our third analysis we evaluated the relationship between spawning characteristics and female attributes. Specifically, we calculated correlations between a female's spawning characteristics (initiation and duration of spawning; number of batches; interbatch interval; average egg size; and batch, seasonal, and relative fecundities) and measures of female status (length, weight, condition, and growth during the spawning season).

Lastly, we assessed the seasonal trends in egg sizes from each female during the course of spawning. For each female, the set of batch-average egg sizes was inspected for linear and curvilinear trends with day-of-year by applying a sequence of first through third order polynomial regressions.

Results

Population patterns (pooled data)

Spawning began in late December and continued until the middle of June (Fig. 1). Most of the batches and most of the eggs were released in the latter half of this period. The median day of spawning in terms of numbers of eggs released was May 7. The batch fecundity was 66 023 ± 52 881 (mean ± SD) and tended to increase as the season advanced (correlation between batch fecundity and day-of-year, $r = 0.31$, $n = 73$, $p = 0.007$).

Egg diameters averaged 1.57 ± 0.092 mm and ranged from 1.28 to 2.02 mm ($n = 2054$). The relative variation (CV = 100(SD/mean)) of the egg size distribution was 5.9%. The statistical distribution of egg diameters departed slightly from normality, as evidenced by a mild positive skewness (i.e., mode < the median). The dispersion of egg diameters was adequately characterized by a lognormal distribution (parameters $\mu = 0.45$ and $\sigma^2 = 0.003$, Fig. 2).

Daily average egg sizes showed no trend with season

Fig. 1. Overall frequency (—) and cumulative (---) distributions of eggs collected from captive Atlantic cod, *Gadus morhua*, during the course of an entire season of spawning.

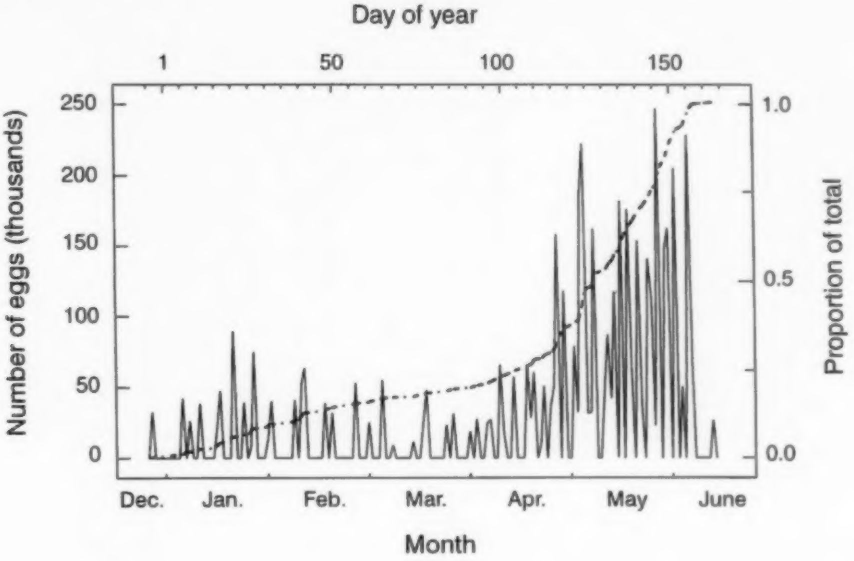
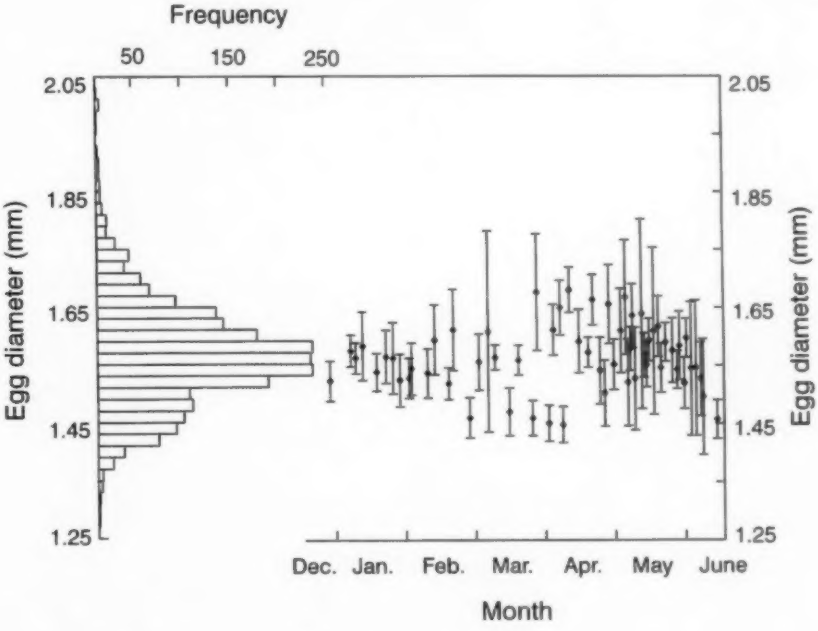


Fig. 2. Overall size–frequency distribution (histogram) and seasonal trend in egg sizes of captive Atlantic cod, *Gadus morhua*, during the course of an entire season of spawning. Diameters in seasonal display are daily means (±SD) for eggs pooled from 10 females.



(evaluated with first- through third-order polynomials; Fig. 2). The average relative variation of the daily egg size was 3.6%, but it fluctuated widely (CV range 1.4–10.8%). The changes in daily variability are a consequence of a variable number of females (one to three) being used to compute the daily statistics.

Female-level patterns

Spawning characteristics

The duration of the spawning period and the number and frequency of batches varied considerably among the 10 females

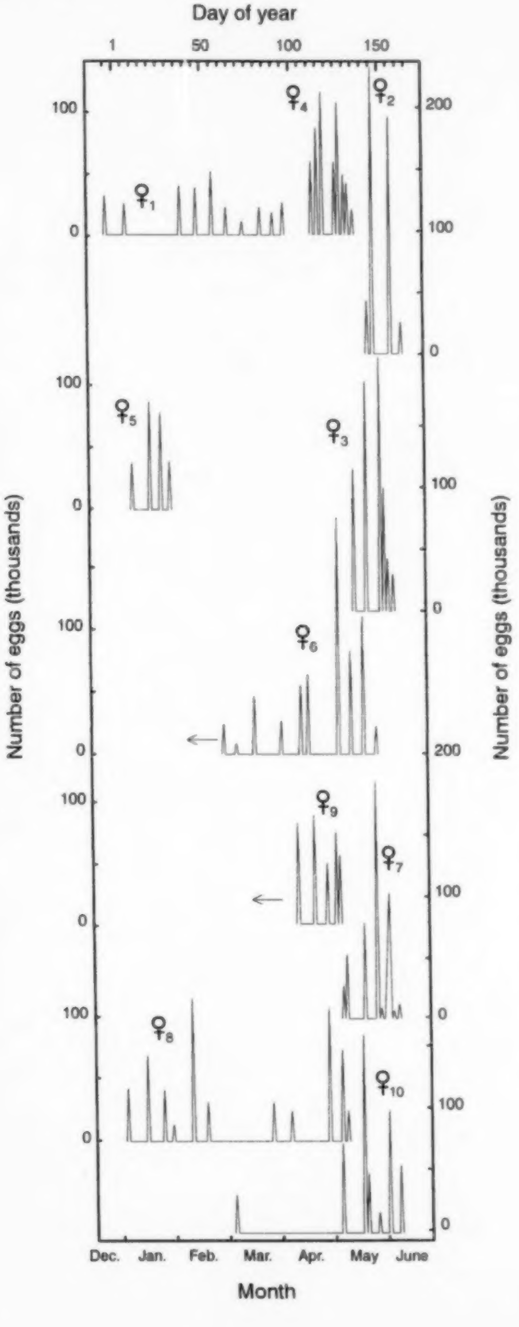
Table 1. Spawning characteristics of captive Atlantic cod, *Gadus morhua*.

Female number	Length (TL, cm)	Weight (wet, g)	Post-spawning condition (100(weight)/ TL^3, $g\cdot cm^{-3}$)	Spawning period initiation (day of year): duration (days)	No. of egg batches	Inter-batch interval (median, days)	Batch fecundity (no. of eggs per batch)	Seasonal fecundity (no. of eggs spawned)	Relative seasonal fecundity (no. of eggs per g female wet. wt.)	Egg diameter (mm)	CV of egg diameter (%)	Change in egg size (%)	No. of eggs measured
1	55.0	2210	0.77	361[a]: 101	10	9	29 255	292 254	244	1.49	3.5	−4.9	287
2	60.6	2150	0.91	143: 20	4	6	126 184	504 739	250	1.50	3.0	−3.8	113
3	59.8	2250	1.00	133: 24	6	3	115 577	693 462	335	1.51	5.2	−11.6	178
4	54.5	2150	1.27	112: 25	8	3	68 345	546 762	267	1.54	7.4	1.0	232
5	53.5	1810	1.08	10.22	4	5	61 781	247 127	134	1.56	3.4	−2.5	114
6	57.5	2250	0.90	60: 86	10	7	64 343	643 432	376	1.57	2.8	−0.8	271
7	59.5	2450	0.99	125: 32	8	3.5	60 220	481 767	218	1.60	4.4	0.0	224
8	67.5	3890	1.10	5: 122	11	8	52 116	573 283	156	1.62	5.6	3.8	296
9	56.5	2090	1.01	99: 25	5	6	70 144	350 720	189	1.63	3.8	−5.7	144
10	45.5	1640	1.63	64: 92	7	6	69 413	485 892	266	1.65	5.0	−11.2	195
Mean±SD	57.0±5.70	2410±612	1.06±0.240	75±56.1: 55±40.2	7±2.5	5.6±2.06	71 738± 28 639	481 974± 146 107	243±74.8	1.57±0.0058	4.4±1.41	−3.6± 5.01	205± 68.1
Pooled data				361,[a] 167	73		66 023± 52 881	4 819 738		1.57±0.092	5.9		2.054

Note: Summary statistics are by female (Nos. 1–10), means over the females, and for pooled data (without regard to female origin). Female numbers reflect an ascending rank based on their mean egg sizes for the entire season of spawning (numbers correspond to those in Figs. 3–5). Female lengths and weights were taken prior to the spawning season. All fecundity estimates are based on the number of eggs released and collected. The change in egg sizes during the spawning season, change in egg size, is based on the deviation of the average egg size of the last batch of the spawning period from the average of the first batch. The number of eggs measured per batch ranged from 22 to 36.

[a] Spawning began on December 27 of previous year.

Fig. 3. Number of eggs collected from each of 10 female captive Atlantic cod, *Gadus morhua*, during the course of an entire season of spawning. The average batch size was ≈72 000 and the largest exceeded 235 000. The egg frequency distributions for individual females are displaced vertically for viewing convenience (arrows denote associated ordinate scale). The numbering system for females is an ascending ranking based on average egg size per female (Nos. 1–10; numbers correspond to those in Table 1 and Figs. 4 and 5).



(Table 1, Fig. 3). The spawning duration averaged 55 ± 40.2 days, the number of batches released averaged 7 ± 2.5, and the median interval between batches was 5.6 ± 2.1 days. Females that began spawning later in the year had shorter spawning durations (day of initial spawning vs. spawning duration, $r = -0.71$, $p = 0.02$) and had smaller intervals between successive batches (initial spawning day vs. interbatch interval, $r = -0.64$, $p = 0.05$). The frequency and duration of spawning by some females, specifically the spawning hiatus in females No. 1, 8, and 10, strikes us as unusual if not abnormal (Fig. 3). Our use of median rather than mean interbatch intervals in our analyses, however, mitigated effects of these spawning patterns. We did not detect an association between intermittent spawning and female status or egg size.

The average batch fecundity per female varied fourfold and averaged 71 738 ± 28 639 over all females. No trend was evident between batch fecundity and the day-of-year of spawning for any female; however as mentioned above, those females late to initiate spawning had a shorter total spawning period. The effect of this shorter spawning period on a female's seasonal fecundity was offset by a higher batch fecundity (initial spawning day vs. batch fecundity, $r = 0.77$, $p = 0.01$). The seasonal fecundity per female varied nearly threefold and averaged 481 974 ± 146 107. The relative fecundity for the entire season also varied about threefold and averaged 243 ± 74.8 eggs per gram female wet weight (Table 1).

The initiation and duration of spawning; number of batches; interbatch interval; and batch, seasonal, and relative fecundities were all independent of female size. Relative fecundity increased with seasonal fecundity ($r = 0.68$, $p = 0.03$), but this is a consequence of the lack of a female size–fecundity relationship and a narrow range of sizes in our females, i.e., among females of the same size, those with greater seasonal fecundities also have greater relative fecundities.

Female and batch effects on egg size

Egg sizes varied significantly among females and among batches-within-females (Table 2, Fig. 4). The variance component of egg size attributable to female identity exceeded that resulting from batches-within-females (35 vs. 26% of the observed variance, respectively), and their sum was over 1.5× the residual, within-batch level (39%). Within each of the 10 females, the fraction of the egg size variance due to batches averaged 40 ± 21.4% and ranged from 16 to 77%. The CVs for egg size within females averaged 4.4%, and those within-batches averaged 3.2%.

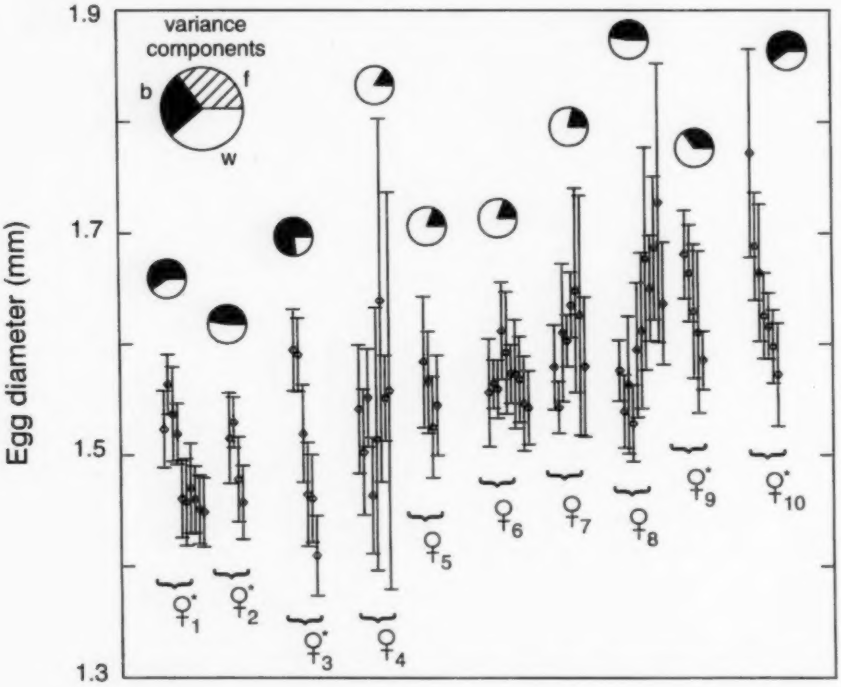
The female effect on egg size was not related to the size of the female per se. Neither length nor mass of the female at the beginning or end of the spawning season was predictive of average egg size for the entire season. Average egg size per female was, however, directly related to female condition (Fulton's *k*). The ranking of females, in terms of their condition, tended to be maintained from the beginning to the end of the spawning season (prespawning vs. postspawning condition, $r = 0.78$, $p = 0.008$). The relationship between female condition and egg size was particularly evident for the first batches of eggs in the spawning period (average egg size of first batch vs. female condition at the beginning ($r = 0.65$, $p = 0.04$) and end ($r = 0.74$, $p = 0.01$) of the spawning season). Additionally, those females that grew the most in

Table 2. Analysis of variance (ANOVA) table and estimated variance components of egg sizes in captive Atlantic cod, *Gadus morhua*.

Source	df	MS	*F*	*p*	Variance component ($\times 10^{-3}$)	Contribution to total variance (%)
Females	9	0.276	203.3	0.001	1.227	35
Batches within female	63	0.027	19.8	0.001	0.911	26
Within batches	1981	0.001			1.360	39

Note: A nested random-effects ANOVA was performed on log-transformed egg diameters. The *F*-ratio for presence of variance due to females is evaluated using the batch mean square as the denominator. The variance components were estimated from the ANOVA method using the Satterthwaite approximation to synthesize the batch mean square and df.

Fig. 4. Egg sizes of captive Atlantic cod, *Gadus morhua*, by batches within each of 10 females during the course of an entire season of spawning. The numbering system for females is an ascending ranking based on average egg size per female (Nos. 1 to 10; numbers correspond to those in Table 1 and Figs. 3 and 5). The egg sizes from batches (mean ± SD diameters) within each female are displayed (left to right) in accord with their seasonal succession. The egg size data for each female are displaced horizontally for viewing. Asterisks indicate significant decreasing trends in average egg sizes across the spawning season, which occurred for 5 of the 10 females. The overall components of egg size variance due to females (*f*), batches (*b*), and within-batches (*w*) were 35, 26, and 39%, respectively (large pie diagram, see Table 2 for statistical tests). The fraction of the egg size variance within each female due to batch effects averaged 40% (shaded areas in small pie diagrams).



length during the spawning season had larger average egg sizes ($r = 0.78$, $p = 0.008$).

Seasonal trends of egg size within females

The batch effect was due mostly to seasonal declines in average egg size per batch (Table 1, Fig. 4). The reduction in mean egg diameters between the first and last batches averaged 4% among all females and was as large as 12%. Smaller females displayed greater seasonal reductions in egg size (female TL vs. change in egg diameter, $r = 0.62$, $p = 0.05$).

A seasonal trend of declining egg sizes was significant in 5 of the 10 females (Fig. 4). For another female (No. 5), the negative correlation between batch-average egg size and day-of-year was large ($r = -0.84$), but a small sample size of $n = 4$ batches precluded statistical significance. Of the remaining four females, one (No. 6) displayed a significant parabolic relationship with egg size: an initial increase in egg size followed by a decline across the last seven batches. Two other females (Nos. 7 and 8) exhibited increasing trends of batch-average egg size during their spawning period.

Discussion

Compared with cod in natural, unexploited populations, the female cod used in this study were of small sizes but sizes that are typical of first- and-second year spawners. These age groups constitute the majority of mature individuals in cod stocks at the present. The narrow range of female sizes, more so than small sizes per se, is likely to have limited our ability to detect differences in spawning characteristics among females, but numerous female effects were nevertheless evident. The cod in this study differed substantially in egg sizes, displayed significant batches effects, and exhibited general though variable declines in egg sizes over the course of the spawning period.

We expect that the phenotypic variation in egg sizes of wild cod would be greater than that reported here because environmental factors and maternal phenotypes are considerably more variable in nature. This expectation is supported by three types of comparisons: (1) the variability of eggs collected on few versus many days, (2) the variability of eggs collected from few versus many females, and (3) the variability of eggs collected from captive versus wild fish. We can make the first two comparisons with our data, and can evaluate the third by comparing our data with literature values. The average daily CV of egg size that we observed was 3.6%, and the average within-female CV for the entire spawning season was 4.4%, both of which are less than the observed overall CV of 5.9% for sizes of eggs pooled across all 10 females for the entire season. This overall CV is, in turn, less than that for cod eggs collected by Miller et al. (1995) in each of two spawning seasons from natural populations on the Western and Sable Banks of the Scotian Shelf (7.5 and 6.8% for two different years) and less than the CV of 7.4% calculated for the cod eggs from the Scotian Shelf reported by Markle and Frost (1985). Although the variability of egg sizes in natural populations appears to be somewhat higher than what we report for broodstock, we anticipate a greater ability to predict egg sizes in nature than was possible with our data, primarily because the effects of seasonality are likely to be clearer in nature (see below). We draw the following provisional conclusions from our analyses.

Overall spawning characteristics and egg sizes

The duration of the spawning season that we report is reasonably consistent with that for natural populations of the region notwithstanding our use of constant temperatures and a narrow size range of females. Cod eggs have been collected from October through June on the Scotian Shelf, Bay of Fundy, and Gulf of Maine (Fish 1930; McKenzie 1934, 1940; Fish and Johnson 1937; Markle and Frost 1985; Miller et al. 1995). Although multiple stocks are included in this region, evidence exists that the spawning season in some stocks can last 8–9 months (Fish 1930; Brander and Hurley 1992; Miller et al. 1995).

Egg size in cod varies among stocks (Table 3). This may reflect intrinsic differences among stocks or may be indicative of a plastic response to the local environment (Chambers 1997). Our estimates of the average and variability of egg sizes are comparable with those reported for eggs of wild cod from local stocks (Bay of Fundy, western Scotian Shelf). Previous comparisons of egg sizes of captive or broodstock cod with those from the parent wild stocks have also revealed egg size to be a conservative feature of a stock (e.g., Solemdal and Sundby 1981; Kjsebu 1989). Eggs of cod from the western Scotian Shelf are similar in size to those of cod from the southeast Barent Sea, and these are exceeded in size only by eggs from cod of the central and eastern Baltic Sea. The larger diameters of Baltic cod eggs, though lighter dry weights (Schopka 1971), are due in part to low salinities typical of the Baltic (Solemdal 1967). Large eggs there, however, also appear to be an adaptive response because largeness enhances egg buoyancy, which increases the likelihood of eggs remaining above the anoxic conditions prevailing at greater depths in the Baltic (Solemdal 1967; Nissling and Westin 1991). As mentioned earlier, the relative variability of egg sizes we report (CV = 5.9%) is less than estimates from field-collected cod eggs from the Scotian Shelf, but the variability of cod eggs is generally high compared with the majority of marine fishes; the median within-population CV of egg diameters for 56 species of marine teleost was 3.8% (Chambers and Leggett 1996). We suggest that the rather large CVs for cod egg sizes are due to the varied environmental conditions experienced by cod during its protracted spawning season and the potentially wide range of ages, sizes, and conditions of females contributing to the pool of eggs. This interpretation is indirectly supported by comparisons among cod stocks. Egg size variation in cod from the Scotian Shelf, at least as reported in studies that have sampled the majority of the 8- to 9-month spawning season, is generally greater than that for cod stocks with shorter (1- to 2-month) spawning seasons (Table 3).

Based on earlier accounts of egg sizes in cod and many other marine fishes (Ehrenbaum 1905, 1909; Sivertsen 1935; Wiborg 1960; Grauman 1965; Rijnsdorp and Jaworski 1990; Marteinsdottir et al. 1993), we expected a seasonal decline in egg size when we considered the pooled collection of eggs. The lack of obvious seasonality in sizes of eggs in our pooled data may be due to our use of constant temperatures, but it also could have resulted from differences among females in their average egg sizes, the degree of seasonal decline in their egg sizes, and the time of initiation and duration of their spawning. These among-female differences, and particularly the effect of asynchronous spawning of females, could blur any pattern of seasonal changes in size of the pooled set of eggs. Such effects were revealed in our data when the average daily egg sizes were decomposed into the contribution of batches from separate females. For example, when eggs from female No. 1, which had the smallest eggs and began spawning first, were excluded from the analysis, a population-level seasonal pattern became evident (Fig. 5).

Table 3. Summary of literature values for egg sizes of Atlantic cod, *Gadus morhua*, from the western and eastern North Atlantic.

Atlantic region	Stock	ICES or NAFO area	Site	Egg diameter (mm)	CV (%)	*n*	Egg source[a]	Comments[b]	Source of data
Western	W Greenland	1		1.42[c]			p	Apr.–May; years; ++stn	Hansen 1968 (p. 129); Andersen et al. 1994 (p. 30)
	Labrador – Grand Bank	2J3KL	Labrador	1.42	3.99[d]	16	p	June 7; 1 stn	Fish 1930 (Table 13)
			Labrador, Newfoundland	1.45[c]			p	Apr.–May	Postalaky 1967 (p. 141)
	S Grand Bank	3NO	Grand Bank	1.28[e]	4.49[d]	489	p	June 5–17; 7 stn	Fish 1930 (Table 13)
	N Gulf of St. Lawrence	3Pn4RS	E Anticosti Island	1.31[f]	4.41	103	p	June–Aug.; 2 stn	Dannevig 1919 (p. 21)
	Banquereau, Sable	4VsW	Western, Sable Island Banks	1.47	7.48	384	p	Oct.–May; 2 years; ++stn	Miller et al. 1995 (p. (1087)
	Banquereau, Sable, W Scotian Shelf	4VsW +4X	Scotian Shelf	1.39	7.38[g]	227[g]	p	Nov.–July; 5 years; ++stn	Markle and Frost 1985 (Table 3, Fig. 3)
	W Scotian Shelf		S Nova Scotia	1.36	4.86[d]	3145	p	Sept.–Dec.; 3 years; ++ stn	McKenzie 1940 (p. 113)
		4X	SW Nova Scotia	1.50	4.10[d]	7251	p	winter–spring; 1 year; ++stn	McKenzie 1940 (p. 113)
			E Bay of Fundy	1.56[c,h]			p	Jan.–?; ++stn	Fish and Johnson 1937 (p. 260–61)
			NW Bay of Fundy	1.42[c,h]			p	spring–Oct.; ++stn	Fish and Johnson 1937 (p. 260–61)
			Passamaquoddy Bay	1.57	5.87	2054	b	Dec.–June; 10 fem	This study
	Gulf of Maine	5Y		1.46			s		Begelow and Welsh 1925 (p. 428)
			Boars Head, Massachusetts	1.43[i]			s	Apr.; 2 fem	Fish 1930 (p. 292)
			Massachusetts Bay	1.49[i]			p	Dec.–June; 1 year; ++stn	Fish 1930 (Table 14)
	S New England	5Zw	Narragansett Bay, Rhode Island	1.36[i]			p	Dec.–May; 2 years; 4 stn	Herman 1958 (cited in Hardy 1978, p. 241) Herman 1963 (103)

Table 3 (*continued*).

Atlantic region	Stock	ICES or NAFO area	Site	Egg diameter (mm)	CV (%)	*n*	Egg source^a	Comments^b	Source of data
Eastern	White Sea	1E		1.58^c			s/b?		Makhotin et al. 1984 (p. 107)
	NE Arctic	1&2	Murman coast, SE Barents Sea	1.50^f			p?		Rass 1936 (p. 253)
			Motovski Bay, SE Barents Sea	1.44^i			p?	Mar.–May	Pertzeva 1920 (cited in Rass 1936, p. 253)
		1&2	Lofoten, N Norway	1.41^g	4.39^g	2017	p	Mar.–Apr.; 4 years; ++ stn	Solemdal and Sundby 1981 (Fig. 3, Table 1)
				1.42^j	4.24	706	p	Apr–; 7 stn	Wiborg 1960 (Table 2)
Eastern	NE Arctic/coastal		Bergen, S Norway	1.29^g	$6.38^{d,g}$	240	s	12 fem	Knutsen and Tilseth 1985 (Fig. 1)
	Iceland	11	Faxafloi, SW Iceland	1.31	4.74^d	94	p	Apr.–May; 1 year; ++ stn	Einarsson and Williams 1968 (Table 5)
			Faxafloi, SW Iceland	1.43	6.94^d	23	p	Apr.–June; 7 years; ++ stn	Einarsson and Williams 1968 (Table 6)
			Selvogsbanki, SW Iceland		1.44^g		s	Mar.–May; 51 fem	Marteinsdottir et al. 1993 (Fig. 6)
	W Scotland	6		1.42^g			s	Feb.–Mar.; 3 fem	Hislop 1984 (Fig. 1)
	W Scotland & North Sea	6 + 4		1.36			s	Feb.–Mar.; 16 fem	Hislop and Bell 1987 (Table 1)
	North Sea	4	W North Sea	1.39			s		Dannevig 1895 (p. 151)
			North Sea	1.41^i			p	Jan.–Apr.	Ehrenbaum 1905–09 (p. 225)
			German Bight	1.41^j	4.37	630	p	Jan.–Apr.; 2 years	Heinke and Ehrenbaum 1900 (App. Table 11)
			Southern Bight	$1.25^{i,k}$			s	Jan.–Mar.; 2 years; 30 fem	Oosthuizen and Daan 1974 (Table 7)
			Southern Bight	1.42^g	5.09^g	1300	p	Jan.–Mar.; 1 year; ++ stn	Rijnsdorp and Jaworski 1990 (Table 1, Fig. 2)
			S North Sea	$1.30^{5,k}$			p	Jan.–Mar.; ++ stn	Rijnsdorp et al. 1991 (Fig. 18)
			Central North Sea	1.33^k			s	Jan.–Feb.; 1 year; 24 fem	Schopka 1971 (Table 27)

Table 3 (*concluded*).

Atlantic region	Stock	ICES or NAFO area	Site	Egg diameter (mm)	CV (%)	n	Egg source[a]	Comments[b]	Source of data
	Irish Sea	7A	Irish coast	1.38			p		Holt 1893 (cited in Heincke and Ehrenbaum 1900 p. 244)
	Skagerrak	3A	S Norway	1.43[j]	4.98[j]	1506[j]	o	Feb.–Apr.; 1 year	Sivertsen 1935 (Table 1)
			S Norway	1.33			?	Apr.	Dannevig 1930 (Table 9?A)
			S Norway	1.37[j,l]			p	Mar.–May; 1 year; ++ stn	Dannevig 1930 (Tables 1, 3, 4, 6, 8, 10, 12, 14, 16, 21, 23, 26)
	Baltic	3D	Belt Sea, W Baltic	1.46[i]			p	Mar.–May	Ehrenbaum 1905–09 (p. 226)
			Belt Sea, W Baltic	1.32[j]	4.67[j]	107[j]	p	Apr.–May; 1 year; 1 stn	Kramp 1913 (Table 1)
			Belt Sea, W Baltic	1.39[i]			p	Mar.; 2 yr; 8 stn	Kramp 1924 (Table 8)
		3D	Bornholm basin	1.70[i]			p	May–Aug.	Ehrenbaum 1905–1909 (p. 226)
			Bornholm basin	1.72[i]	4.21[i]		p	Mar.–Aug.; 2 years; ++ stn	Grauman 1965 (Table 1, 2)
			Bornholm basin	1.74[g]	6.44[g]	1317	p	May–June; 2 years; ++ stn	Müller and Bagge 1984 (Fig. 4, 5)
			Gdansk basin	1.75[i]			p	Apr.–Aug.; 2 year; ++ stn	Grauman 1965 (Table 1)
			Gotland basin	1.75[i]			p	Apr.–June; 2 years; ++ stn	Grauman 1965 (Table 1)
			Gotland basin	1.66			s	May–June; 3 years	Nissling et al. 1994 (p. 69, Fig. 3)

Note: Priority was given to field-based estimates, either from ichthyloplankton surveys or eggs stripped from ripe females. Egg size from broodstock are in general agreement those from source populations. Stock names and areas follow ICES and NAFO conventions (Brander 1994*b*). Site detils from original report are included if more specific than stock names. Values given are those in the original report except where noted.

[a] Egg source: p, eggs collected from plankton; s, eggs stripped from ripe wild females; b, broodstock; o, ripe ovarian eggs.

[b] Entries in comments field: date, months, season, years of egg collection; number of stations (stn) sampled (++, many); number females (fem) from which eggs were obtained.

[c] Midpoint of range given.

[d] CV based on SD estimated from range and n (Sokal and Rohlf 1981, p. 58; Rohlf and Sokal 1969, Table Z).

[e] Weighted mean of values given.

[f] Eggs listed as cod and haddock eggs are likely to have been cod (Dannevig 1919); all observations used here to compute statistics.

[g] Statistics estimated from figure.

[h] Assumed to be cod by Fish and Johnson (1937).

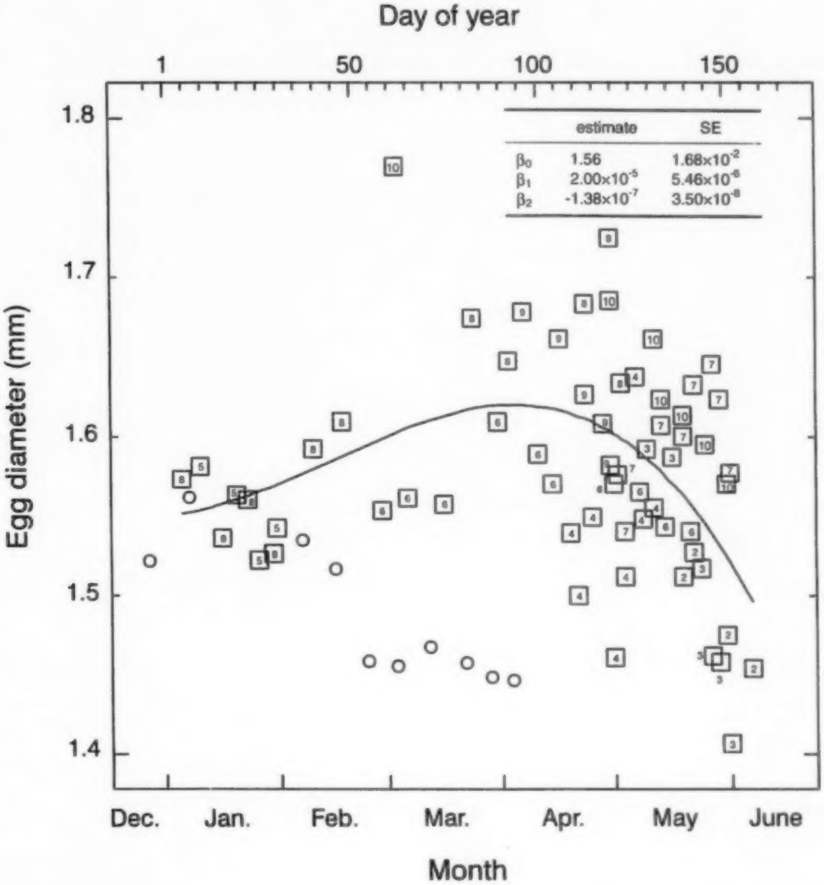
[i] Mean of values given.

[j] Statistics based on frequency table given.

[k] Egg diameter estimated from egg dry weights (mg) using expression (from data in Hislop and Bell 1987): egg diameter = 0.988 + 3.548(egg dry weight).

[l] Egg size computed under our assumption that cod and haddock eggs were in equal abundance when collected together.

Fig. 5. Seasonal trend in average egg sizes in egg batches from 10 captive Atlantic cod, *Gadus morhua*, during the course of an entire season of spawning. The numbers within each symbol denote the female number (as per Table 1, Figs. 3 and 4). The predicted values (—) are based on the polynomial: egg size = $\beta_0 + \beta_1\,(\text{day})^2 + \beta_2\,(\text{day})^3$ computed using the daily average sizes of eggs pooled from all females except for female No. 1 (○). All parameter estimates (inserted table) are significant at the 0.001 level.



	estimate	SE
β_0	1.56	1.68×10^{-2}
β_1	2.00×10^{-5}	5.46×10^{-6}
β_2	-1.38×10^{-7}	3.50×10^{-8}

Spawning characteristics at the female level

Although the duration of the spawning season for our captive population is comparable with local populations (167 days) and long relative to eastern North Atlantic stocks, the spawning durations for individual females (mean 55 days) are similar to values reported for Norwegian coastal cod. Kjesbu (1989) reported spawning durations of 47, 51, and 60 days for three Norwegian cod females experiencing warmer temperatures than our 4°C regime. The first of his females was held indoors at 8°C, and the latter two were held outdoors at ambient temperatures (5–7°C). In contrast, the average number of batches that we report (seven) is fewer by half, and the interbatch interval (5.6 days) is 1–2 days longer than that observed for Norwegian cod. The cod used by Kjesbu (1989) were larger than ours (mean TL 67 vs. 57 cm), and his were of considerably higher condition prior to spawning (mean k = 1.5 vs. ours at 1.2) and higher than wild Norwegian coastal cod ($k \approx 1.0$). Not only are our averages for these reproductive characteristics different from those given for Norwegian cod by Kjesbu and colleagues, but the variability among females in initiation and duration of spawning, total number of batches, and interbatch intervals that we report is also greater. Differences in the sizes and levels of condition of females could be responsible for some of these discrepancies. Also, as mentioned earlier, the frequency and duration of spawning by some of our females appears abnormal relative to values reported for Norwegian cod. However, inasmuch as substantial phenotypic differences are evident in egg sizes and spawning seasons from one cod population to another, we cannot dismiss the potential for stock-specific differences in other reproductive characteristics. A more extensive evaluation of spawning season duration and batch frequencies in fish from local stocks is forthcoming (E. A. Trippel, Biological Station, St. Andrews, N.B., unpublished data).

The seasonal fecundities that we observed are comparable with those reported for cod of similar lengths in western North Atlantic stocks. Estimates of (ovarian) fecundities from field-collected cod from the Canadian Atlantic are available for stocks from Grand Bank – Labrador (May 1967) and southern Gulf of St. Lawrence (Buzeta and Waiwood 1982). May (1967) reported fecundities to range from 0.2 million to over 11 million for females of lengths from 50 to 127 cm length. From May's fecundity–length equation, a female equal in length to our average (57 cm) would have approximately 506 000 eggs (cf. our average fecundity of ≈482 000). Fecundity estimates for a 57-cm female derived from equations for southern Gulf of St. Lawrence cod range from 369 000 to 631 920 (Buzeta and Waiwood 1982). These estimates are higher than expected for like-sized Northeast Arctic cod and coastal Norwegian cod (≈393 000 and 493 000, respectively; Brander 1994*a*). Mass-specific fecundities in our study are lower than those predicted for females from the southern Gulf of St. Lawrence, Grand Bank – Labrador, Icelandic, Norwegian, North Sea, and Baltic stocks. These estimates of length- and mass-specific fecundities are point estimates based on power relationships between fecundity and female size (summarized in Oosthuizen and Daan 1974; Buzeta and Waiwood 1982). As point estimates, these predicted fecundities should be treated cautiously in general and all the more so here because our females were at the low end of the size range used in the original parameter estimation. Furthermore, all of the fecundities used to derive these relationships were based on ovarian egg counts, which are higher than realized fecundities.

Female and batch components of egg size variation

This study is unique in simultaneously evaluating the female and batch contributions to egg size variability for the entire spawning season. Our estimates of the variance components of egg size show differences among females to account for over a third of the total variance in egg size and the sum of female and batch components to constitute over 60% of the total variance. One implication of a female effect on egg size is that samples of eggs taken from a female at any time during the spawning season will be predictive of egg sizes at other dates. As a means of quantifying this predictability, we note a direct relationship between the average egg size in the first batch of eggs and the average egg size for the entire season ($r = 0.78$, $p = 0.01$). Egg sizes in the first and last batches of turbot, *Scophthalmus maximus*, show a similarly large direct correlation ($r = 0.67$, $n = 22$, $p = 0.001$; McEvoy and McEvoy 1991). These relationships could be used to improve egg production estimates and to identify preferred individuals for broodstock or culture. We interpret the batch effect as a seasonal trend in egg sizes, rather than haphazard differences in egg sizes from batch to batch, because females that exhibited the greatest batch effects are also those with the largest directional changes in egg sizes across the spawning season (females Nos. 1–3 and 8–10; Fig. 4).

We expect that the magnitudes of variance components for egg sizes in nature would fluctuate among years and among sites because of environmental variability and changes in the status of spawning females. Unfortunately, we know of no other study that reports variance components of cod egg sizes to compare with ours, nor of any study of variance components of egg sizes or other life-history traits in any other batch-spawning marine fish. Egg size differences among female cod have been demonstrated numerous times (e.g., Solemdal 1970; Schopka 1971; Oosthuizen and Daan 1974; Hislop 1984; Knutsen and Tilseth 1985; Nissling and Westin 1991; Nissling et al. 1994), but the female and batch (seasonal) effects cannot be separated in these data.

In lieu of comparison with other studies on cod or other batch-spawning marine fishes, we compared our estimates of female and residual variance components with those for two other species of marine fish, but whose females spawn for a short period each year. The female variance component for egg sizes in capelin, *Mallotus villosus*, an osmerid that is typically semelparous and sheds all eggs within several days, was estimated as 71% (Chambers et al. 1989). In winter flounder, *Pleuronectes americanus*, an iteroparous pleuronectid that sheds all eggs within several days to several weeks, 46% of the variance in egg sizes was attributable to female effects (Chambers and Leggett 1996). These values are higher than the female variance component for cod (35%), perhaps because the capelin and flounder females in those studies were obtained by sampling natural populations and, therefore, may be more representative of the full range of maternal phenotypes than is true for our captive cod. Further experiments with a broader variety of species is necessary to determine whether the disparities among these studies in their estimates of variance components of egg size reflect study designs, species differences, or more general differences in reproductive tactics.

We know of no additional estimates of variance components for egg sizes in other marine fishes, although substantial female differences in egg sizes have been reported for populations of Atlantic herring, *Clupea harengus*, Pacific herring, *Clupea pallasi*, Atlantic silverside, *Menidia menidia*, European flounder, *Platichthys flesus*, flathead sole, *Hippoglossoides elassodon*, haddock, *Melanogrammus aeglefinus*, mummichog, *Fundulus heteroclitus*, striped bass, *Morone saxitilis*, turbot, and walleye pollock, *Theragra chalcogramma* (reviewed in Chambers 1997; Chambers and Leggett 1996). Female effects on egg sizes have also been reported for freshwater fishes (Marsh 1984; Lagomarsino et al. 1988; Kamler 1992) and for a number of freshwater and anadromous species under aquaculture (Tave 1993). Indeed, we think it noteworthy that no study known to us has failed to detect differences in egg sizes among females when observations were made at the appropriate level.

Associations with female status

Period of spawning

Female size was not predictive of spawning duration in our data, but numerous studies that have used a wider range of sizes (and ages) of cod provide evidence that larger, older females initiate spawning earlier and spawn for longer than smaller, younger females (Sund et al. 1938; Wiborg 1960; Solemdal 1970; Jónsson 1982; Marteinsdottir et al. 1993). The fact that our largest female (No. 8) began spawning early and had the greatest spawning duration is suggestive of an age effect. This pattern has also been reported for other marine fishes, e.g., European plaice, *Pleuronectes platessa* (Simpson 1956), red tilefish, *Branchiostegus japonicus* (Hayashi 1977), and Atlantic herring (Lambert and Messieh

1989). The evidence is often indirect; larger, older fish dominate the catches from the spawning grounds early in the spawning season, and the ages and sizes of ripe females decline as the season proceeds. But this pattern is also supported by data on age-specific seasonal changes in gonadosomatic indices of cod (Berner 1985). The conclusion that larger, older females spawn first and for longer is not without exceptions, at least for cod. Analysis of oocyte maturation stages of cod from waters off Newfoundland and Labrador led Hutchings and Myers (1993) to conclude that younger fish initiate and complete spawning earlier than older fish. Recent laboratory investigations on Norwegian coastal cod also suggest that larger cod initiate spawning later but spawn for longer than smaller cod (Kjesbu 1994), although this effect is slight and may be obscured by variability among individual females. In nature, the female age effect may be compounded with a temperature effect because age-specific migration patterns of females may expose females of different ages to different temperature regimes during oogenesis (Kjesbu 1994).

Fecundity

Neither age, size, nor condition of a female was predictive of batch, seasonal, or relative fecundities in our cod, but fecundity is known to increase with size when a broad size range of cod is considered (Powles 1958; May 1967; Schopka 1971; Oosthuizen and Daan 1974; Buzeta and Waiwood 1982). Part of the difficulty in detecting an influence of female condition on fecundity may result from the separation between the time when condition (or physiological processes related to it) influences fecundity and the time that condition and fecundity are estimated (Woodhead and Woodhead 1965). For example, the size-at-age and growth of cod in summer (June to September) appear to influence the potential fecundity of cod in the following spring (Waiwood 1982).

Egg size

Egg size did not increase with female size; indeed, the shortest and lightest female in our study produced the largest eggs (Table 1). Moreover, a study subsequent to the present investigation, but which used some of the same females, found egg diameters to average 1.58 ± 0.120 mm ($n = 182$). This egg size compares favourably with the average diameter of 1.57 mm reported here, even though the females were on average over 50% longer (90 ± 3.8 cm vs. 57 ± 5.7 cm TL) and 5 years older at the time of the second study (R.C. Chambers, unpublished data). Results from other studies are in disagreement regarding relationship between egg size and female size or age. Egg size appears to increase with female size in Baltic cod (Grauman 1964, 1973; Schopka 1971), but no relationship was evident in North Sea and Icelandic stocks (Schopka 1971) when evaluated over a wide range of female sizes, e.g., North Sea cod from 60 to 105 cm (Oosthuizen and Daan 1974). Kjesbu (1989) reported a direct relationship between female size and the average egg size in the first batch of eggs of the spawning season, but such a relationship was not evident in our data for female length ($r = -0.52$, $p = 0.13$) or weight ($r = -0.38$, $p = 0.28$). Kjesbu et al. (1992) also found egg size to increase with female age, though the age effect they report is less than the female size effect on egg size in the same females. No age effect on egg size was detected in Northeast Arctic cod (Solemdal 1970), but Grauman (1973) reported an increase in egg size with age of female Baltic cod.

We view the direct relationships between egg size and a female's condition after spawning, as well as female growth during the spawning season, as evidence of the importance of the amount of energy available to a female for reproduction. Along these same lines, Grauman (1965) reported that egg sizes in Baltic cod were larger and contained more lipid in years when females experienced high prey levels in the previous autumn. However, there does not appear to be a consistent relationship between female condition, or level of ration, and egg size in cod or other marine fishes. Hislop et al. (1978) provided evidence of the positive effect of female ration on egg size in haddock, but commented that this effect is inconsistent across species. Kjesbu et al. (1991) reported that egg sizes in their captive cod did not seem affected by high ration levels; egg sizes of captive cod were comparable with egg sizes from local wild cod despite a 1.5× greater average level of condition in their captive cod. The commonly reported inverse relationship between somatic lipid levels and the energy invested in gonadal tissues (Dambergs 1964; Jangaard et al. 1967; Love 1970; Shevchenko 1972) supports the general connection between storage lipids and oogenesis but detecting a condition effect on egg size may require more direct and sensitive measures of condition. Indeed, Kjesbu et al. (1991) argued that egg sizes in a batch are ultimately determined by the energy available in the several-day interval between the last and next egg batch.

The likelihood of detecting a relationship between egg size and female status in wild fish seems low given the inconsistent evidence of a link between egg size and female age, size, and condition and the small magnitude of effects when they do occur. For purposes of quantifiying such relationships, we recommend studies that monitor the entire spawning intervals of cod of a broad range of ages, sizes, and conditions. More obvious differences among females in their reproductive characteristics have been reported for comparisons between first-time (recruit) spawners and repeat spawners. Reduced spawning durations, lower fecundities, and smaller eggs have all been reported for recruit spawning cod (Earll 1880; Jónsson 1982; Ellertsen and Solemdal 1990) and other marine fishes (Hempel and Blaxter 1967; Hislop 1988), and these differences may prove to predominate among the possible maternal influences. Considering all reproductive females, careful accounting for population, year, female, and seasonal effects are needed to resolve the discrepancies among the results of prior studies.

Seasonal trends of egg size within females

In our study the sizes of eggs shed by a female generally decreased as the spawning season advanced. Similar decreases in egg sizes over the course of spawning have also been noted in captive Norwegian coastal cod held at constant temperatures (Kjesbu 1989; Kjesbu et al. 1991, 1992), but decreases were not evident in individual Baltic cod monitored for a period covering three to five consecutive egg batches (Nissling et al. 1994). We calculated the extent of seasonal change in egg diameters for nine females reported in the Norwegian studies by Kjesbu and colleagues and found an average seasonal decline of 7% (range 0–15%). Considering the entire population of eggs in our study, and those of Kjesbu et al. and Nissling et al., the seasonal changes in the sizes of eggs from captive cod appear to be less dramatic and less consistent than those reported for natural populations (e.g., Erhenbaum 1905, 1909; Sivertsen 1935; Wiborg 1960; Solemdal 1970; Marteinsdottir et al. 1993). We interpret this contrast as evidence that a different combination of factors is affecting seasonal changes in egg sizes in nature.

Various mechanistic and adaptive explanations have been advanced to account for the changes in egg sizes over the course of the spawning season in cod and other marine fishes. Three of the more common are (1) an inverse relationship between egg size and temperature; (2) a direct relationship between egg size and female age, coupled with older females spawning earlier in the spawning season; and (3) a direct relationship between egg size and female condition, coupled with decreasing condition of the female as the spawning season advances. Neither the explanation due to temperature nor age can be evaluated with our data because we used constant temperatures and females of similar ages.

Our data are consistent with the explanation based on female condition. Specifically, a direct relationship was evident between egg size in the first batch and female condition measured either before or after spawning. Moreover, females that grew most in length and lost the least weight during the spawning season also had larger eggs. Our cod seldom fed during spawning, which is not uncommon among winter–spring spawning fishes of temperate and boreal latitudes, so we can assume that energy reserves are decreasing during the spawning period. Because the final size of cod eggs before they are shed appears to be determined in the several-day interval between the most recent and next batch of eggs (Kjesbu et al. 1991), the production of smaller eggs later in the spawning season may be particularly characteristic of batch-spawning species whose females rely on stored energy for the final stages of oogenesis. Grauman's (1965) report of larger eggs in Baltic cod in years when females experienced high prey levels in the prior autumn is consistent with this interpretation.

The less dramatic and less consistent seasonal decreases in egg size in studies of captive cod relative to the patterns evident in nature suggest that additional factors are indeed operative in wild populations. Evidence of an age effect on seasonal changes in egg size is equivocal. As mentioned earlier, a direct relationship between female age (or size) and egg size either has not been detected (Solemdal 1970; Schopka 1971; Oosthuizen and Daan 1974) or is of low magnitude (Kjesbu et al. 1992). Moreover, the evidence that older (larger) females spawn earlier than younger (smaller) ones is also inconclusive (Sund et al. 1938; Wiborg 1960; Solemdal 1970; Jónsson 1982; Marteinsdottir et al. 1993; Hutchings and Myers 1993; Kjesbu 1994).

There is substantial evidence of an inverse relationship between temperature and egg size in cod and other marine teleosts (Chambers 1997). Indeed, the diversity of spawning times among cod stocks offers special insights into the potential for such temperature effects. Stocks of cod that spawn in the spring (with rising temperatures) consistently show seasonal declines in egg sizes, e.g., Icelandic (Marteinsdottir et al. 1993), Northeast Arctic (Sivertsen 1935; Wiborg 1960; Solemdal 1970), and most stocks of the Baltic (Grauman 1965) and North Sea cod (Erhenbaum 1905, 1909; Rijnsdorp and Jaworski 1990). Cod spawn in the autumn in some stocks of the Baltic Sea (Berner 1985), North Sea (Brander 1994*b*), and western North Atlantic Ocean (McKenzie 1940; Brander and Hurley 1992). Still other stocks in the western North Atlantic spawn from autumn into early summer (Fish 1930; Miller et al. 1995). Fish (1930) reported that cod eggs collected on the spawning grounds in Massachusetts Bay, southern Gulf of Maine, U.S.A., increased in size through the autumn, reached a maximum size near the time of lowest temperatures (February), and then decreased to a minimum size by the time that spawning stopped in June. Recent year-round ichthyoplankton collections on the Scotian Shelf corroborate the inverse relationship between cod egg size and water temperature (Miller et al. 1995).

Indeed, autumn-spawning cod stocks provide critical information for assessing the above three explanations of seasonal changes in egg sizes. By including data from autumn spawners, the general pattern now to be explained is that eggs decrease in size from winter to summer and increase in size from autumn to winter. While the temperature explanation is fully consistent with the revised pattern, both the female-age and female-condition explanation require fundamental modifications. Specifically, the female-age explanation must be amended to include the possibility that, in the autumn, older females either spawn large eggs late in the season or spawn small eggs early. The female-condition explanation must be altered to allow for the possibility that eggs increase in size as a female's energy reserves decrease (we assume that a female's energy reserves decrease as the spawning season advances regardless of time of year). These modifications appear to substantially weaken the generality of a female-age or a female-condition explanation of seasonally varying egg sizes.

Conclusions

The sizes of young larvae of marine fishes are influenced by egg size and by the environmental conditions experienced during the embryonic period (Chambers 1997). Both types of influences are likely to be associated with the time and location of spawning, which may in turn be related to the age and size of the female and her condition during oogenesis. Female effects have long been suggested to be operative in cod and other species (Heinke and Ehrenbaum 1900) but heretofore have received little quantitative analysis. The potential importance of maternal effects is underscored by recent findings that individuals that survive to recruitment come from distinct subsets of the entire range of spawning dates for a year-class (Al-Hossaini et al. 1989; Noichi et al. 1994; Rose et al. 1996). Given the variation among females in spawning characteristics and egg sizes, it is likely that recruits are likewise produced by a subset of all spawning adults.

Further studies are necessary to determine the extent to which egg sizes and other spawning characteristics depend on female status and the environment and whether these characteristics are more broadly representative of stock differences. This determination has significant utility for fisheries management in light of the assumption made in some assessment models that spawning stock biomass is a sufficient descriptor of the numbers and quality of eggs produced by the stock (Trippel et al. 1997). We believe that an experimental approach offers a profitable means of analyzing many of these issues; indeed, our findings of significant female and batch effects would have been difficult to obtain in any other way.

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Effects of hatchery and wild ancestry and rearing environments on the development of agonistic behavior in steelhead trout (*Oncorhynchus mykiss*) fry

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Abstract: At emergence, fry from a wild steelhead trout (*Oncorhynchus mykiss*) population displayed higher levels of one type of mirror-elicited agonistic behavior (swimming against a mirror) than did fry from a locally derived hatchery (domesticated) population. Newly emerged wild fry also dominated hatchery fry in size-matched dyadic dominance challenges. However, given an approximately 3.0–4.5% size advantage, hatchery fry dominated smaller wild competitors in 68% of encounters, indicating that small size differences at emergence can strongly influence dominance. Hatchery fry reared in a natural stream channel for 105 days were more aggressive than those reared in hatchery tanks and than wild fry reared in either environment. In otherwise identical hatchery tanks, low density and low food rations were associated with elevated agonistic behavior by hatchery fry, but not by wild fry. This study suggests that four to seven generations of domestication has resulted in behavioral divergence of the hatchery population from its wild donor population. The extent to which such differences determine the outcomes of interactions between offspring of wild and hatchery steelhead spawning in streams will depend on the size differences and emergence dates of the populations as well as genetic bases of aggression.

Résumé : Chez les alevins récemment éclos d'une population sauvage de truites arc-en-ciel (*Onchorhyncus mykiss*) anadromes, on a observé qu'un type de comportement agonistique suscité par un miroir (les sujets attaquent le miroir en nageant) est plus marqué que chez des alevins provenant d'une écloserie locale (population domestiquée). Les jeunes alevins sauvages ont également dominé ceux de l'écloserie dans des provocations de domination dyadiques. Toutefois, compte tenu d'un avantage de taille d'environ 3,0 à 4,5%, les alevins de l'écloserie ont dominé les compétiteurs sauvages de plus petite taille dans 68% des rencontres, ce qui indique qu'une faible différence de taille à l'éclosion peut avoir une grande influence sur la dominance. Les alevins de l'écloserie qui ont été élevés dans un cours d'eau naturel pendant 105 jours étaient plus agressifs que ceux qui ont été élevés dans les bassins de l'écloserie et que les alevins sauvages élevés dans l'un ou l'autre de ces milieux. Dans des bassins semblables, on a observé que lorsque la densité et les rations alimentaires sont faibles, le comportement agonistique est marqué chez les alevins de l'écloserie, mais non chez les alevins sauvages. Cette étude indique qu'après quatre à sept générations de domestication, la population de l'écloserie présente une divergence de comportement par rapport à la population sauvage dont provenaient les géniteurs des sujets de l'écloserie. L'influence de ces différences sur l'issue des interactions entre les alevins de truites arc-en-ciel anadromes sauvages et les alevins élevés en écloserie lorsqu'ils fraient dans des cours d'eau dépend des différences de tailles et de l'époque de l'éclosion de même que des facteurs génétiques de l'agressivité.
[Traduit par la Rédaction]

Introduction

Hatchery programs for anadromous salmonids can supplement natural production but may have negative ecological consequences for wild populations (see Steward and Bjornn 1990 for a review). In particular, juvenile hatchery salmonids may have competitive advantages over wild conspecifics (Chandler and Bjornn 1988; Swain and Riddell 1990) that could contribute to the displacement of wild fish (Nickelson et al. 1986). Stream-dwelling juvenile salmonids compete for resources such as food and cover through agonistic behavior (Chapman 1962; Hartman 1965). Levels of aggression have been positively associated with dominance ability (Egglishaw 1967; Fenderson and Carpenter 1971; Holtby et al. 1993), and winners of dominance encounters generally obtain the most energetically profitable stream positions (Fausch 1984; Metcalfe 1986).

Swain and Riddell (1990) demonstrated that newly emerged coho salmon (*Oncorhynchus kisutch*) fry from populations that had been cultured for several generations (i.e., domesticated) were more aggressive than fry from geographically proximate wild populations. Agonistic behavior has a heritable component in coho salmon (Riddell and Swain 1991), but differences in agonistic behavior among wild coho and chinook salmon (*Oncorhynchus tshawytscha*) populations

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also exist and apparently have a genetic basis (Taylor and Larkin 1986; Rosenau and McPhail 1987). Thus, studies comparing the behavior of a wild population to that of hatchery-produced fish from another population may be confounded. The present study compares agonistic behavior of a wild steelhead population to a locally derived hatchery population, thereby minimizing the potential for interpopulation differences not related to domestication.

Variation in rearing environments can also affect agonistic behavior of juvenile salmonids. For example, food deprivation increased levels of agonistic behavior in juvenile chum salmon (*Oncorhynchus keta*; Olla et al. 1990) and Atlantic salmon (*Salmo salar*; Symons 1968). In other studies on Atlantic salmon, agonistic activity of naturally reared fry was less than that of hatchery-reared fry when both groups were held at high densities, but the reverse was true at the lowest densities (Fenderson et al. 1968; Fenderson and Carpenter 1971).

Relative body size has also been recognized as a strong determinant in the outcome of dominance encounters for over 30 years (Chapman 1962). The interplay, however, between body size and aggressiveness has been studied recently (Huntingford et al. 1990; Holtby et al. 1993) but remains unclear. We investigated the relationship between aggression, relative size, and dominance by first evaluating between-population differences in levels of aggression, and then evaluating contests between socially naive individuals of equal and unequal size from the different populations.

Behavioral characteristics at any developmental stage result from present and past interactions between an individual's genotype and external environmental factors (Alcock 1993). Genetically based and environmentally induced effects of hatcheries on aggressive behavior of salmonids have been investigated by controlling for either genotype or rearing environment, respectively. However, we have limited understanding of how environmental factors influence the development of agonistic behavior for hatchery and wild populations that have innate behavioral differences.

Naturally produced steelhead (*Oncorhynchus mykiss*) in the Pacific Northwest typically live in streams for 2 or 3 years prior to seaward migration, whereas hatchery-produced steelhead are most commonly released at age 1. To "push" hatchery-reared steelhead to reach a threshold smolt size (Wagner 1968; Seelbach 1987; Tipping et al. 1995) in 1 year, they are typically fed very high rations. They are also reared at high densities to maximize the production potential of hatcheries. The high rations and densities common in hatcheries contrast sharply with conditions encountered by wild steelhead in natural streams. Fry-to-smolt mortality is much greater for wild than for hatchery-reared fish, and wild fish may survive better at sea than hatchery fish. The different selection regimes encountered by wild and hatchery populations may cause adaptive, genetic changes over a few generations of culture.

The objectives of this study were to investigate the effects of hatchery ancestry, rearing environment, and their interaction on the development of agonistic behavior in juvenile steelhead and to determine the relative effects of aggression and size-at-emergence on the outcome of between-population dyadic dominance encounters. Specifically, we tested the following null hypotheses: (1) levels of agonistic behavior in newly emerged steelhead fry did not differ between hatchery and wild populations; (2) population type, rearing environment, and their interaction had no effect on the development of agonistic behavior of age–0 fry; and (3) population type and body size had no effect on dominance interactions between newly emerged fry.

Materials and methods

Study populations

Fertilized eggs were obtained from one-to-one matings of wild Quinault River, Washington, steelhead on March 22, 1993 (six pairs), and March 31 to April 2, 1994 (seven pairs). Steelhead were collected in the southwest corner of Lake Quinault and were identified as wild by the absence of the fin damage ubiquitous in Quinault Tribal Hatchery steelhead (M. Figg, Quinault Indian Fisheries Division, Amanda Park, Wash., personal communication). Subsequent scale analysis indicated that these steelhead had migrated to sea after 2 years in freshwater and had narrowly spaced freshwater circuli, also indicating natural rearing (Leider et al. 1990). Scales of one male and one female spawned in 1993 could not be definitively analyzed because of scale regeneration, but both fish were used because their fin condition and run timing were similar to wild Quinault steelhead.

Fertilized eggs were also obtained from one-to-one matings of hatchery steelhead spawned at Quinault National Fish Hatchery on Cook Creek, a tributary to the lower Quinault River, on March 23, 1993 (8 pairs), and February 15 and 23, 1994 (10 pairs). The Cook Creek population was derived solely from wild adult steelhead captured in the mainstem of the lower Quinault River from 1972 to 1974. There is no evidence that steelhead were ever introduced to the Quinault National Fish Hatchery or Quinault River system from another river system. Cook Creek steelhead are released as 1-year-old smolts and spend from 2 to 4 years in the ocean. Hence, those spawned in 1993 and 1994 were as many as four to seven generations removed from their wild ancestors. Scale analysis showed that the Cook Creek brood fish had migrated to sea after 1 year in freshwater and had wide, uniform spacing of freshwater circuli. Both of those traits indicated that these fish were hatchery reared as juveniles.

Reisenbichler and Phelps (1989) suggested that Lake Quinault may serve as a partial barrier to gene flow between the upper and lower river that allowed the upper Quinault population to genetically differentiate from the lower Quinault and other populations along the Washington coast. They also found that Cook Creek steelhead had allozyme frequencies distinct from wild Quinault steelhead and other Washington coastal populations but concluded that these differences were an artifact of the hatchery program and did not necessarily indicate adaptive differences. We believe that the wild broodstock collected in Lake Quinault for this study are the closest to the donor wild population available, and that comparing Cook Creek hatchery and Quinault wild populations provides a valid evaluation of the effects of domestication on steelhead fry behavior.

Rearing environments

Eyed eggs were transported to the University of Washington's Big Beef Creek Research Facility, Kitsap County, Washington, and incubated on well water in Heath trays. In 1993, eyed eggs from both stocks were incubated at 10°C until emergence. In 1994 incubation temperatures were manipulated to synchronize emergence of both populations and to facilitate the sequence of mirror image stimulation experiments (MIS) and dominance challenges. In 1994, Cook Creek eggs were incubated at average temperatures ranging between 5.6°C and 7.2°C and wild Quinault eggs, between 8.3°C and 10.0°C, depending on whether they were to be placed into rearing tanks, or used in dominance challenges or MIS experiments.

With the exception of fry that would be tested at the button-up stage of development (i.e., when the yolk sac was no longer externally exposed), fry from both populations were released into hatchery and natural environments in 1993. Four treatment combinations of population and rearing environment were created: CN, CH, QN, QH, where C is Cook Creek (hatchery) population, Q is Quinault (wild) population, H is hatchery environment, and N is natural environment.

The natural rearing environment consisted of seven consecutive 14.9-m^2 riffle–pool habitat units on each side of a wooden barrier (total of 14 units) in a side channel of Big Beef Creek. The units were separated from one another by wood-framed screens constructed of 8 mm square mesh galvanized screen, which prevented fry from moving between the units. The channel received a water flow of approximately 0.06 $m^3 \cdot s^{-1}$ from the main channel of Big Beef Creek. Red alder (*Alnus rubra*), western cedar (*Thuja plicata*), and salmonberry (*Rubus spectabilis*) comprised the main canopy and streamside vegetation. Fish were exclusively dependent on natural production of terrestrial and aquatic invertebrates. Food production in the stream channel has been shown to be very similar between channels and among units (Spalding et al. 1995). Steelhead rearing in the stream channel were vulnerable to kingfishers (*Ceryle alcyon*), green-backed herons (*Butorides striatus*), and blue herons (*Ardea herodias*), but no piscivorous fish were present. Each unit contained one sunken Douglas fir tree top, approximately 1.5 m in height, for cover. One hundred thirty-eight newly emerged Cook Creek fry were stocked into each of seven randomly chosen riffle–pool units, and each of the remaining seven units were stocked with 138 Quinault fry. There was equal representation from each family within their respective population.

The hatchery environment consisted of dark green, square rearing tanks (1.8 × 1.8 m wide, 0.5 m deep), supplied with well water routed through a heat exchanger to induce diel temperature fluctuations similar to those in the stream channel. Fry were stocked at an initial density of 2839 $fish \cdot m^{-3}$ and were scatter-fed a semimoist crumble before being switched to a dry feed after 2 weeks. The fish were initially fed six times daily, which was gradually reduced to twice daily over the 105-day rearing period. The food ration was decreased from 4.5% to 3.0% of biomass over the course of the study, approximating the food manufacturer's guidelines for satiation feeding.

In 1994, offspring from both populations were reared only in hatchery tanks to determine if the two most obvious differences between the stream channel and the hatchery tanks in 1993 (food availability and rearing density) would affect both populations equally. Emergent fry from each population were divided among four rearing tanks so that each tank received equal representation from all families. Two of the four tanks were stocked at a “high density” (D) of 2469 $fish \cdot m^{-3}$ and two at a “low density” (d) of 235 $fish \cdot m^{-3}$. One tank at each density was fed a “high ration” (R), reduced from 6% of biomass to 4% of biomass from emergence to 59 days post-emergence and the other a “low ration” (r), reduced from 2.5 to 1%. Fish biomass was estimated weekly by individually weighing a sample of 50 fry from each tank, and food rations (weighed daily to the nearest 0.1 g per tank) were adjusted accordingly. This procedure was followed for both populations, creating a total of eight tanks with all combinations of population (Quinault and Cook Creek), density (high and low), and ration (high and low). All tanks received 23 $L \cdot min^{-1}$ of 10°C well water throughout the study.

Mirror image stimulation

Mirror image stimulation (MIS) experiments (Gallup 1968) were conducted to quantify agonistic behavior. MIS tests allow for adequate replication of subjects and have the advantage over dyadic or group encounters of matching a fish against an opponent of equal size and motivational state and eliminating bias from differential agonistic responses to kin and nonkin (Brown and Brown 1992, 1993). Agonistic responses of coho and chinook salmon fry to MIS correlated with responses to conspecifics (Taylor and Larkin 1986; Rosenau and McPhail 1987; Taylor 1988; Swain and Holtby 1989). Although there has been some debate as to the applicability of MIS experiments (Ruzzante 1992), they have been used successfully to differentiate levels of aggressiveness among populations (Holtby and Swain 1992). We used MIS to simultaneously evaluate population and rearing environment effects on agonistic behavior of steelhead.

Mirror-elicited behavior in salmonids has been categorized into three aggressive behaviors (swim against mirror (SAM), lateral display (LAT), and wigwag (WIG) and one submissive behavior (SUB). Definitions of these behaviors follow those described in MIS studies involving coho and chinook salmon (Taylor and Larkin 1986; Rosenau and McPhail 1987; Swain and Riddell 1990; Riddell and Swain 1991; Holtby et al. 1993). SAM was a swimming motion by a fish with its snout against the mirror, often involving a violent shaking of the head and snapping of the jaws. Lateral display included a range of threat displays with the fish parallel to the mirror, with dorsal and anal fins erect, mouth open, and body stiffened. The fish remained motionless or paddled forward with lowered pectoral fins. Lateral displays while swimming with exaggerated body motions were categorized as WIG displays. WIG and LAT were measured separately but were combined for analysis. Submissive displays met all of the following criteria: a fully depressed dorsal fin, depressed anal fin, and a partially folded caudal fin.

All MIS experiments, described below, were conducted in 19-L aquaria, situated in two, 10 × 1.2 m wooden flumes. Each flume was divided in half longitudinally, creating four channels with 10 Plexiglas viewing windows (41.0 cm wide × 30.5 cm high) per channel. The aquaria were placed inside the flumes so that one aquarium could be seen through each of the 40 observation windows. Water was recirculated through the flumes at a depth of 27 cm (just below the tops of the aquaria) to provide excellent visibility between the viewing windows and the aquaria. Each aquarium received 0.5 $L \cdot s^{-1}$ of 10.5°C well water sprayed through a 3 mm mesh screen that covered the top. Gravel substrate was added to the aquaria to a depth of 3–4 cm.

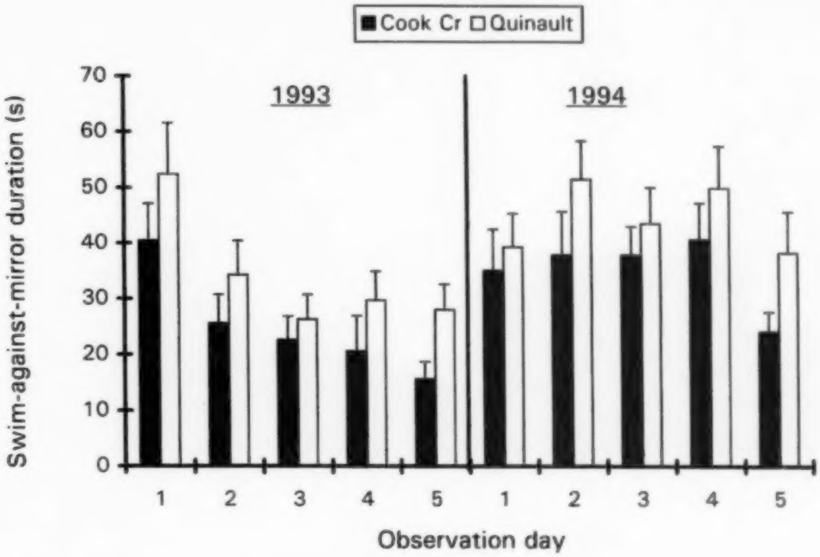
During daylight hours an intensity of 600 lux illumination at the water surface was provided by five pairs of wide-spectrum 40W fluorescent bulbs over each flume. Light intensity during nighttime was reduced to 30 lux, provided by fluorescent bulbs approximately 3.0 m from the sides of the flumes. The flumes were covered with black plastic sheets to avoid disturbing the fish during observation.

In 1993 MIS experiments were conducted at successive developmental stages: emergence (i.e., at button-up; EM-93), 30 days postemergence (PE30-93), and 105 days postemergence (PE105-93). In 1994, experiments were conducted at emergence (EM-94) and 51–59 days postemergence (PE51-94). Thus, during the 2 years there were five experiments. Socially naive fry tested at emergence (EM-93 and EM-94) were removed from the incubation trays, measured (fork length), and placed individually into the aquaria. Each family within a population was equally represented (±1 fish) to avoid bias due to potential among-family differences in social behavior (Quinn et al. 1994). Fry used in the three experiments that involved a rearing phase were randomly selected from their respective rearing environments and placed individually into the aquaria.

For all experiments, fry were fed twice daily during a 4-day acclimation period, then once at least 1 h after testing on observation days. All fish had begun feeding by 48 h prior to each experiment. Fry were fed commercial salmon feed during EM-93, EM-94, and PE51-94. However, during PE30-93 and PE105-93 fry were fed frozen thawed bloodworms (*Tubifex* spp.) because half of these subjects were reared in the natural stream channel and preliminary revealed that they would not readily eat commercial pellets. Bloodworms appeared to be equally acceptable to all groups.

Experiments in 1993 involved 40 or fewer trials so all treatment

Fig. 1. Daily average (±SE) swim-against-mirror (SAM) durations for socially naive, newly emerged fry from the wild Quinault and Cook Creek hatchery populations in 1993 and 1994. The two populations differed significantly in levels of SAM ($n = 38$ per population, $p = 0.04$).



groups were tested simultaneously. Experiment PE51-94 involved more subjects than there were available tanks so observations had to be conducted in two sessions because only 40 aquaria were available. Two sets of trials were conducted such that 40 fry (5 from each of the 8 tanks) were tested beginning at 51 days postemergence and 32 fry (4 from each tank) were tested beginning at 59 days postemergence.

Each trial was initiated by placing a mirror over the entire back of the aquarium so that the fish could see its reflection from anywhere in the tank. After 45 elapsed, the 3-min observation period began. The observer was unaware of the fish's treatments group (with the exception of EM-94) to eliminate potential observer bias. The duration of each behavior pattern was recorded on a notebook computer. Each fish was observed once daily for 6 days in all 1993 experiments and 5 days in both 1994 experiments.

The dependent variable was the duration of each behavior (SAM, LAT, or SUB) during 3 min of observation. Data from all MIS experiments were first rank transformed to correct for heteroscedasticity, then analyzed by a nested-factorial ANOVA to incorporate the repeated measures on individual subjects over the course of each experiment (Hicks 1987; Tabachnick and Fidell 1989). Individual subjects were nested within populations and years for the combined analysis of EM-93 and EM-94. Subjects were nested within populations and rearing environments for PE30-93 and PE105-93 and within population, density, and ration for PE51-94. This design allowed us to test for (1) the main effects of population and year, (2) the effects of population and rearing environment (hatchery vs. natural) in 1993, (3) the effects of population, ration and density in 1994, and (4) all interactions. In addition, all main effects and interactions in each of the five experiments were crossed by observation day, which provided tests for the effect of observation day and observation day by other main effects interactions.

Dominance challenges

Dominance challenges were conducted in 12 nonadjacent chambers (64 cm long × 61 cm wide × 27 cm deep) situated in the flume channels. The upstream and downstream barriers of each chamber were constructed of fine-mesh nylon netting. A layer of gravel (0.5–2.0 cm in diameter) covered the bottom of each chamber to a depth of 5.0 cm. Water was recirculated through the flumes creating a flow of 8.4 $L \cdot s^{-1}$ through each chamber. Sixteen trials were conducted at emergence where one socially naive fry from each population was paired with a fry of equal fork length (±0.25 mm measurement error) from the other population. In subsequent trials, also conducted at emergence, fry from the "subordinate population" (determined by equal fork length trials 1 through 16) were given a 1.0 mm ($N = 14$) or 1.5 mm ($N = 14$) fork length advantage over a competitor from the dominant population. To identify the two fish in the chambers, adipose fins were clipped on one or the other fry so that each population received adipose clips in half of the trials. Fish that were not adipose clipped were handled in a similar manner including rubbing of scissors across the adipose fin to simulate a clip.

Each trial was begun by placing a pair of fry into a chamber and allowing them to acclimate overnight for approximately 16 h. On day 2 a small amount of commercial steelhead food was introduced into the upstream center of a chamber, and then again 30 s later. Agonistic behavior was then observed for 4 min. A second feeding and 4 min observation period was repeated 3–4 h later. This procedure was repeated on day 3 for a total of 16 min of observation over a 30-h period for each trial.

Dominance was assigned on the basis of total nips and chases delivered (Newman 1956; Abbott et al. 1985), the number of times a fish crossed the midline of the tank when not being chased, and the presence of a pale body coloration and prominent parr marks on the dominant fish and a dark lateral band on subordinates (Keenleyside and Yamamoto 1962). The sign test was used to test the null hypothesis that the number of both populations produced the same number of winners in both size-matched and size-mismatched trials.

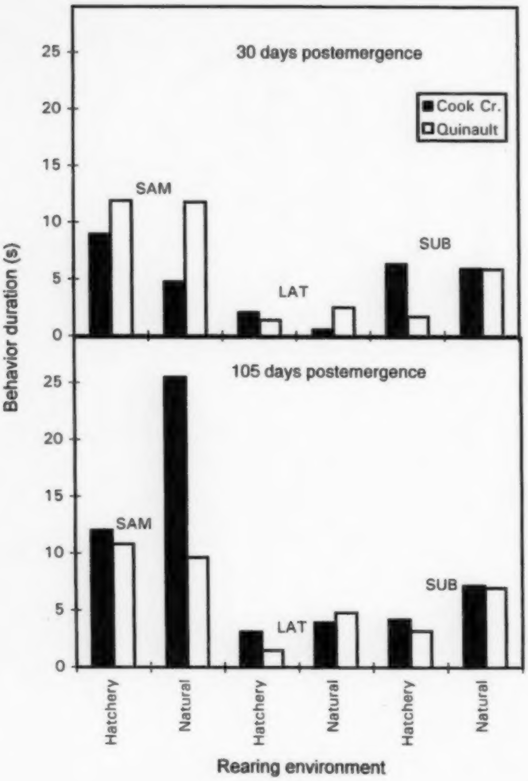
Results

Mirror image stimulation

Population comparison at emergence

At the button-up stage of development (i.e., emergence) wild

Fig. 2. Average swim-against-mirror (SAM), lateral display (LAT), and submissive (SUB) behavior durations by Cook Creek and Quinault fry reared in hatchery and natural environments for 30 or 105 days. No significant day, or day by main effects interactions existed, so each bar represents the average of six observation days. There was a significant (p = 0.004) population by rearing environment interaction for SAM at 105 days postemergence. Both graphs are shown on the same scale to demonstrate the differences between behavior durations at 30 and 105 days postemergence.



Quinault fry displayed greater levels of SAM than Cook Creek fry ($F_{1,72}$ = 4.30, p = 0.04). Swim-against-mirror durations decreased over the 5-day experiment in 1993 for both populations, but levels of SAM showed no apparent trend in 1994 (Fig. 1). The observation day by year interaction was significant ($F_{4,288}$ = 5.96, $p < 0.001$). The observation day by year interaction was also significant for lateral displays ($F_{4,288}$ = 3.43, p = 0.01), and the trends were similar to those for SAM (i.e., decrease over days in 1993 and no apparent trend in 1994). The Quinault and Cook Creek populations did not differ significantly in the duration of LAT ($F_{1,72}$ = 0.13, $p > 0.50$) and only slightly in SUB ($F_{1,72}$ = 3.73, p = 0.06).

Population and rearing effects:1993 results

At 30 days postemergence wild Quinault fry continued to display slightly greater, though not statistically significant, levels of SAM than Cook Creek hatchery fry ($F_{1,36}$ = 1.70, p = 0.220). There were also no significant differences between the populations or rearing environments in levels of LAT or SUB ($p > 0.05$ for all main effects and interactions).

The size distributions and mean sizes of fry from both populations in the stream channel were similar to those in the hatchery tanks at 30 days postemergence. By 105 days postemergence, however, stream-reared fish from both populations had greater variation in weight and fork length and were significantly smaller, on average, than those in the hatchery tanks, suggesting that the stream channel was a food-limited environment at 105 days postemergence but not at 30 days postemergence (Berejikian 1995).

At 105 days postemergence there was a significant interaction between population and rearing environment for SAM duration ($F_{1,180}$ = 7.17, p = 0.001). Cook Creek fry reared in the natural stream channel (CN) displayed much greater levels of aggression than did the other three population–environment combinations (CH, QH, and QN). That is, the stream environment caused an increase in aggression for Cook Creek fry (Scheffe's multiple contrast, $p < 0.05$), but not for Quinault fry ($p > 0.05$). There was no day, or day by other main effects interaction so the data were pooled across observation days to display the nature of the population by rearing environment interaction (Fig. 2). Population type and rearing environment had no effect on LAT and SUB durations at 105 days postemergence ($p > 0.05$ for all main effects and interactions), and the overall durations of these behaviors were similar to those at 30 days postemergence.

Population, ration, and density: 1994 results

There was a significant highest order interaction: day by population by ration by density for SAM duration during PE51-94 ($F_{4,256}$ = 2.97, p = 0.022). The decline in mean SAM duration for Cook Creek, high-density, low-ration fish (i.e., CDr) and concomitant increase for QDr after day 3 appeared to contribute to this interaction (Fig. 3). Possibly, fish adapted their behavior to the test environment after several days. After some period of time in the test environment, behaviour may be more strongly influenced by the test environment then by the pretest treatment. Because of this possibility, we believe that the results on the earlier test days of this experiment more accurately reflected the effects of the pretest environment. We, therefore, re-analyzed the data for the first 3 days of the experiment only.

Over the first 3 days there was no significant day by population by ration by density interaction ($F_{2,128}$ = 0.22, $p > 0.50$). There was a significant population by ration by density interaction for SAM ($F_{1,64}$ = 7.45, p = 0.008). Cook Creek fry reared under high ration and high density (CDR) displayed significantly lower levels of SAM than Cook Creek fry reared under low ration and low density. (Scheffe's multiple contrast $p < 0.05$; Fig. 4), and the levels of SAM displayed by CDR were very similar to those of Quinault fry reared under the same conditions (QDR). The combination of high density and high ration appears to have suppressed the development of aggression within the Cook Creek population. By contrast, levels of SAM for QDR did not differ significantly from those of Quinault fry reared under the other combinations of density and ration (Scheffe's multiple contrast, $p > 0.05$).

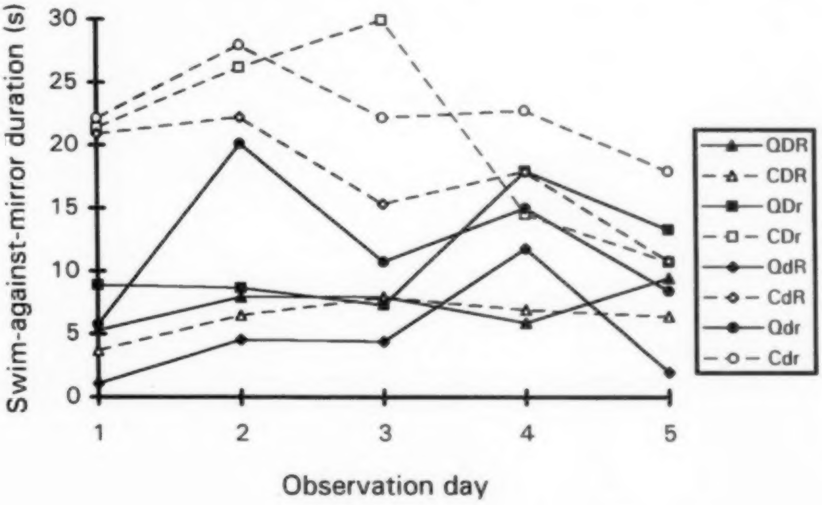
The population by ration by density interaction was marginally significant for LAT ($F_{1,64}$ = 4.05, p = 0.05), and the

Table 1. ANOVA results (days 1 through 3 only) for mirror image stimulation experiment PE51-94 in which Quinault and Cook Creek fry were reared under high and low densities and rations in hatchery tanks to 51 to 59 days postemergence.

	SAM			LAT		SUB	
Effect	df	*F*	*p*	*F*	*p*	*F*	*p*
Population	1,64	9.71	0.002	11.25	<0.001	0.03	—
Ration	1,64	6.14	0.017	4.87	0.032	4.77	0.034
Density	1,64	0.57	—*	1.88	—	0.23	—
Population × density	1,64	1.65	—	2.61	—	0.61	—
Population × ration	1,64	0.64	—	5.02	0.029	0.69	—
Ration × density	1,64	0.64	—	0.12	—	2.69	—
Population × ration × density	1,64	7.45	0.008	4.05	0.050	2.06	—
Day	2,256	1.69	—	0.30	—	2.49	—
Population × day	2,256	0.46	—	0.15	—	0.10	—
Density × day	2,256	1.28	—	0.31	—	0.35	—
Ration × day	2,256	0.79	—	0.02	—	0.38	—
Population × density × day	2,256	0.20	—	0.05	—	0.02	—
Population × ration × day	2,256	0.03	—	0.11	—	4.39	0.016
Density × ration × day	2,256	0.26	—	0.01	—	0.59	—
Population × ration × density × day	2,256	0.22	—	0.13	—	0.12	—

$^*p > 0.10$

Fig. 3. Average daily swim-against-mirror (SAM) durations in experiment PE51-94 for each combination of population (Q, Quinault; C, Cook Creek), density (D, high; d, low), and ration (R, high; r, low). The day by population by density by ration interaction was significant ($p = 0.022$).



lower order population by ration interaction was significant ($F_{1,64} = 5.02$, $p = 0.03$; Table 1). Cook Creek fry reared under low ration, regardless of density, exhibited much higher levels of LAT than Quinault fry under the same conditions (Fig. 4). However, the Cook Creek low-density high-ration group (CdR) exhibited low levels of LAT, whereas this same group displayed high levels of SAM. Thus, for Cook Creek fry, low density may have had less effect on LAT than on SAM, whereas low ration was associated with high levels of both behaviors. There was a significant population by ration by day interaction for SUB ($F_{1,256} = 4.39$, $p = 0.16$).

Dominance

Quinault fry won more dominance bouts (87.5%) than Cook Creek fry (12.5%) when there was no measurable difference in fork length (sign test, $n = 16$, $p = 0.004$; Table 2). In subsequent trials, Cook Creek fry were given a 1 or 1.5 mm (approximately 3.3–4.5%) fork length advantage over

Table 2. Outcomes of dominance challenges between Cook Creek and Quinault fry at emergence.

Cook Creek size advantage (mm)	N	Wins		df	p
		Cook Creek	Quinault		
None	16	2	14	1	0.004
1.0	14	9	5		
1.5	14	10	4		
Overall (1–1.5)	28	19	9	1	0.09

Note: Data from 1.0- and 1.5-mm size-mismatched trials were combined for analysis.

Quinault fry. The magnitude of the size difference had no significant effect on the outcomes of dominance bouts between the two populations (Chi-square test of homogeneity, $p > 0.50$) so the data from 1 and 1.5 mm size difference trials were combined. Larger Cook Creek fry won 68% of the bouts against smaller Quinault fry, which was a nonsignificant difference (sign test, $n = 28$, $p = 0.09$; Table 1). The frequencies of Quinault fry and Cook Creek fry winners, however, depended on whether the fry were size matched (Quinault fry won 87.5%) or size mismatched (Quinault fry won 32.5%). Therefore, the effect of body size was significant for these agonistic contests (Chi-square test of independence, $p < 0.001$). We were unable to detect any effect of adipose fin clips on the outcome of agonistic encounters (sign test, $n = 44$, $p > 0.25$)

Discussion

When paired at emergence, wild Quinault fry won significantly more dyadic dominance challenges than equalsized Cook Creek hatchery fry, consistent with their higher levels of aggression at emergence. Huntingford et al. (1990) conducted within-population dominance experiments with Atlantic salmon and concluded that fighting ability, termed fierceness, may be the primary determinant of dominance and that size was more likely a consequence rather than a cause of dominance. Holtby et al. (1993) also suggested that body size may be a consequence of dominance and found that aggressiveness and lateral body profile were associated with dominance. Our results also suggest that aggressiveness may partly determine dominance because the Quinault population, which had higher levels of SAM, dominated equal sized Cook Creek fry in 87.5% of the contests. However, when given a slight (<5%) fork length advantage, Cook Creek fry dominated smaller Quinault fry in 68% of the contests, indicating that body size at emergence may also be a critical determinant of dominance for steelhead trout. Metcalfe et al. (1995) demonstrated that dominance in newly emerged Atlantic salmon was positively correlated with standard metabolic rate within full-sib groups; however, we did not collect metabolic data to test for such a relation in this study.

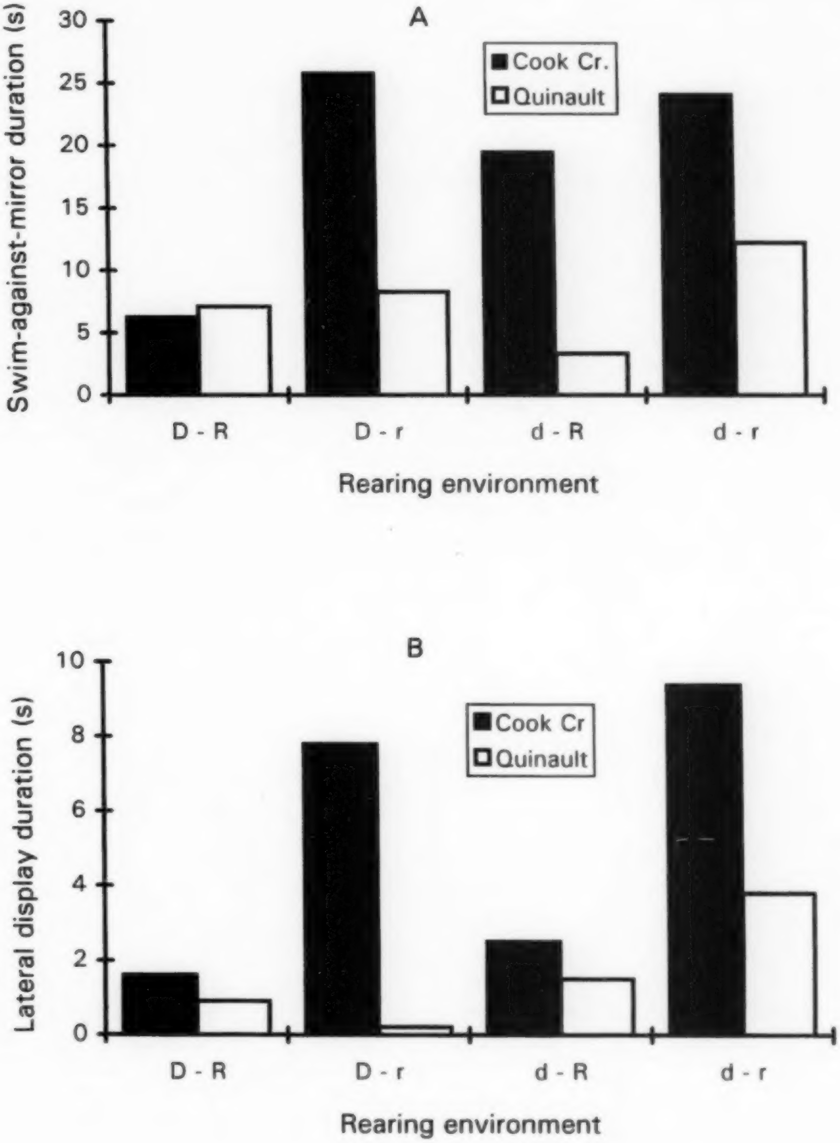
Declining SAM and LAT durations in 1993, and lack of a decline in 1994, might be explained by differences in the amount fed to individual fry between years. During acclimation and testing in 1993 emergent fry were fed slightly more than 10% of their biomass per day whereas in 1994 fry were fed at 7% of body weight (rations in 1994 were weighed individually for each fish to the nearest 0.001 g, much more precisely than in 1993). Fry from both populations tested in 1993 may have become satiated, reducing motivation to engage in agonistic behavior (cf. Symons 1968; Olla et al. 1990).

Cook Creek fry were more aggressive than Quinault fry in both years when reared under low densities and low food availability. At 105 days postemergence in 1993, Cook Creek fry reared at a relatively high ration and density in the hatchery tanks exhibited lower levels of aggression than Cook Creek fry reared under low ration and low density in the stream channel conditions. Similarly, Cook Creek fry reared under low ration and low density were more aggressive than Quinault fry reared under the same conditions. In fact, levels of SAM for Quinault fry were similar regardless of their rearing environment in both 1993 and 1994 (Figs. 2 and 4). Based on "socially naive" fry from the two populations at emergence, (2) the population by rearing environment interactions, and (3) the strong likelihood that the two populations are of common ancestry, we conclude that the observed differences in agonistic behavior between Cook Creek and wild Quinault steelhead have a genetic basis, probably created by four to seven generations of culture in the Quinault National Fish Hatchery.

The lifetime fitness of wild fry may depend more strongly on establishing dominance early in life (i.e., immediately following emergence) than it does for hatchery fry. Dominant individuals gain the most energetically profitable stream positions (Fausch 1984; Metcalfe 1986) and grow faster than subordinates (Newman 1956; Kalleberg 1958; Yamagishi 1962), possibly as a result of increased access to food (Metcalfe et al. 1988; Nielsen 1992). Dominance relationships in juvenile steelhead trout cannot be reversed through size reversals; hence, dominant fish tend to maintain their status (Abbott and Dill 1989), and as a result of their larger size, are probably better able to avoid natural predators than smaller, subordinate fry (Bams 1967; Patten 1977). In protected, food-rich hatcheries, fry are reared under high densities where "scramble competition" (Milinski and Parker 1991) predominates and territories cannot be defended (Grant and Kramer 1990). Although hatchery fry probably also benefit from rapid growth and large size at release (see below), optimal food acquisition strategies are likely to differ for hatchery-produced fry and wild fry emerging in natural streams (see also Doyle and Talbot 1986).

The greater levels of aggression demonstrated by Cook Creek fry under natural conditions (i.e., low rations and densities) may be an indirect result of asymmetrical food and territory requirements of fry from the two populations. As mentioned, hatchery-reared steelhead are fed very high rations to reach smolt size in 1 year. Fish that do not grow large enough to smolt in 1 year are unlikely to emigrate to sea (Tipping et al. 1995) and may reside in freshwater for an additional year or more. Nonmigrating fish are far more likely to die or spawn in the stream rather than return to the hatchery outflow (Wagner 1969; Cramer 1981) and, hence, would not

Fig. 4. Average durations of (A) swim-against-mirror (SAM), and (B) lateral displays (LAT) for PE51-94. Data were pooled across observation days 1–3 to display the nature of the population by ration (R, high; r, low) by density (D, high; d, low) interactions, which were significant for SAM ($p = 0.008$) and LAT ($p = 0.05$).



contribute to the next generation of hatchery fish. Recent data provided no evidence of 2-year freshwater age hatchery-produced steelhead returning to the Quinault National Fish Hatchery (D. Zajac, U.S. Fish and Wildlife Service, Olympia, Wash.). Two-year freshwater age hatchery-reared steelhead were also absent in adult hatchery returns over a 6-year period in the Kalama River, Washington (Loch et al. 1985). Therefore, natural selection should, favor fast-growing fish, which may include those fish that maximize food intake through high levels of aggression but suffer no consequences (e.g., increased vulnerability to predation) in a protected hatchery environment (see Swain and Riddell 1990). If selection for rapid growth is concomitant with increased systemic demand, hatchery steelhead may experience greater levels of hunger than wild fish when provided with limited rations. Hunger is positively associated with aggression (Symons

1968; Olla et al. 1990); thus, the higher levels of aggression displayed by Cook Creek fry under low rations compared with Quinault fry under the same conditions may also reflect differences in food requirements of individuals from the two populations.

Rearing density did not appear to affect levels of agonistic behavior as strongly as ration. Cook Creek fry reared at low density and high ration displayed levels of SAM more than three times as high as those reared under high density and high ration, whereas levels of LAT showed less of a rearing density effect (Fig 4). Grant and Kramer (1990) surveyed existing literature and determined that territorial behavior of several salmonid species breaks down at high densities. Our results suggest that rearing fish at high densities from emergence may also hinder the development of aggression, when food is plentiful.

The consequences of hatchery steelhead straying to spawn with wild steelhead are still unclear. Nickelson et al. (1986) provided evidence that hatchery coho salmon presmolts outplanted along several Oregon coastal streams replaced wild coho but produced no increase in overall adult populations. The concern that hatchery populations may displace wild populations through superior competitive ability has been supported by Swain and Riddell (1990), who found that newly emerged fry of two domesticated coho salmon populations were more aggressive than those of geographically proximate wild populations. Their results suggested a genetic basis for the population differences. Our results support this concern in the following respect. Cook Creek steelhead displayed elevated levels of aggression when reared in two different food-limited environments (a natural stream channel in 1993 and hatchery tanks receiving reduced ration in 1994). Offspring of domestic steelhead that reproduce in the wild would experience even lower food availability than was provided in our experiments and consequently should be more aggressive than offspring of wild spawners by 2 months postemergence, or sooner. The association of aggression with dominance ability for anything but socially naive fry is difficult to demonstrate because prior experience affects success in subsequent agonistic encounters (Francis 1983; Abbott et al. 1985; Beacham 1988). However, in general, levels of aggression are correlated with dominance (Egglishaw 1967; Fenderson et al. 1971), and in particular, SAM can predict dominance status in coho salmon (Holtby et al. 1993). In addition to aggression advantages, offspring of naturally spawning domestic steelhead might have the competitive advantages of prior residence and body size (Chapman 1962; Mason and Chapman 1965; Abbott et al. 1985; Chandler and Bjornn 1988) over offspring of wild steelhead because hatchery fish typically spawn earlier and, therefore, probably hatch earlier.

Although domesticated steelhead may have some competitive advantages over wild steelhead, empirical evidence demonstrates that naturally spawned offspring of domesticated steelhead may suffer greater mortality throughout all life-history stages than offspring of wild steelhead (Reisenbichler and McIntyre 1977; Chilcote et al. 1986; Leider et al. 1990). In addition to the potential fitness benefits of early social dominance, which wild fry demonstrated in this study, genetically based predator-avoidance behaviors may be partly responsible for the poor fry-to-smolt fitness of domesticated populations. For example, Johnsson and Abrahams (1991) found that offspring of wild steelhead × domestic rainbow trout hybrids were more willing than offspring of pure wild steelhead to risk exposure to predation, but hybrids were no better able to survive brief exposure to predation. Cook Creek steelhead fry were less able to survive predation by prickly sculpin (*Cottus asper*) than were wild Quinault fry in two laboratory experiments and one field experiment (Berejikian 1995). Observations made during the laboratory experiments suggest that predator recognition and vigilance may have been important mechanisms.

Finally, the timing of spawning and emergence is an important consideration in determining the relative success of wild and hatchery-origin salmonids in streams. Early timing may facilitate acquisition of territories and provide a size advantage, but selective predation on early emerging fry may reduce this advantage (Brännäs 1995). The combination of potentially superior competitive ability (i.e., high aggression 2–3 months after emergence), and inferior predator avoidance ability of hatchery fry suggests that caution should be exercised in designing hatchery programs to supplement wild populations (see also Reisenbichler and McIntyre 1986; Hindar et al. 1991; Waples 1991).

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Effects of temperature and vaterite replacement on the chemistry of metal ions in the otoliths of *Oncorhynchus tshawytscha*[1]

R.W. Gauldie

Abstract: Chinook salmon (*Oncorhynchus tshawytscha*) were reared from the egg to 297 days under the same experimental conditions and then separated for a further 43 days into five temperature treatment groups each of 100 fish at 8, 10, 12, 14, and 16°C plus a control group for a total of 634 fish. Many otoliths recovered from the experiment had aragonite partially replaced by vaterite. Whole otolith chemistry was measured using inductively coupled argon plasma optical emission spectrometry. The concentrations of the metal ions strontium, magnesium, manganese, sodium, iron, and zinc as well as phosphorus were recorded. The chemistry of the otolith was weakly related to somatic weight, somatic length, temperature treatment, and otolith weight. Vaterite replacement accounted for a large part of the variation observed in metal ion concentrations in the otolith.

Résumé : Nous avons élevé 634 saumons quinnats (*Oncorhynchus tshawytscha*) dans les mêmes conditions expérimentales depuis l'oeuf jusqu'à l'âge de 297 jours, puis les avons séparés en cinq groupes de 100 poissons maintenus pour 43 jours supplémentaires dans des eaux à 8, 10, 12, 14 et 16°C respectivement, plus un groupe témoin. Chez de nombreux otolithes prélevés dans le cadre de l'expérience, l'aragonite était en partie remplacée par de la vatérite. Nous avons établi la composition chimique des otolithes complets par spectroscopie d'émission avec plasma d'argon induit par haute fréquence et avons noté la concentration du phosphore et des ions métalliques (strontium, magnésium, manganèse, sodium, fer et zinc). La composition chimique des otolithes présentait une relation peu marquée avec le poids du corps, la longueur du corps, la température de traitement ainsi que le poids de l'otolithe. La variation de la concentration des ions métalliques dans l'otolithe était en grande partie attribuable au remplacement de l'aragonite par de la vatérite.
[Traduit par la Rédaction]

Introduction

Most teleost fishes have three otoliths (Dale 1976) in each of the pair of endolymphatic sacs of the inner ear. The three otoliths are variously involved in hearing and balance (Fay 1980). The chemistry of the sagitta, the largest of the three otoliths, varies among individuals. Variation in individual otolith chemistry has been described in terms of regional populations (or isolated stock) structure (Edmonds et al. 1989, 1991), ambient water chemistry (Gunn et al. 1992; Mugiya and Tanaka 1995), ambient water temperature (Radtke 1989; Toole and Nielsen 1992; Arai et al. 1995), the internal structure of the otolith, and growth rate and size of the fish (Grady et al. 1989; Sadovy and Severin 1992). The otoliths of fishes often show a complex polymorphism involving the aragonite, vaterite, and calcite polymorphs of calcium carbonate (Gauldie 1993) leading to a situation in which the otoliths of some species may be part aragonite, part vaterite, and part calcite. The chinook salmon, *Oncorhynchus tshawytscha*, is one such species for which otoliths composed of mixtures of aragonite and vaterite have been described (Gauldie 1986; 1991). The effect of rearing temperature on the chemistry of the aragonite otoliths of juvenile *O. tshawytscha* has been described by Gauldie et al. (1986). This paper describes the effects of partial vaterite replacement on the chemistry of the sagittae of juvenile *O. tshawytscha* reared at different temperatures.

The physiological bases for relationships between otolith weight, fish weight, and otolith chemistry have not been established in the literature. The paper follows an empirical statistical treatment of otolith growth and otolith chemistry. Where possible, the least amount of transformation of the data is used, and lacking alternative theoretical models based on physiology, linear regressions and linear partial regressions are used.

Materials and methods

The fish used in the experiment were drawn from a single pair mating of *Oncorhynchus tshawytcha* from wild stocks of the Quinsam River on Vancouver Island, British Columbia, that took place on 27 October 1978. Genetic control was approximated by restricting the experiment to the offspring of a single pair mating. All the fish were subsequently reared together and fed to repletion for the duration of the entire experiment on the same batch of Oregon Moist Pellet. Mortality past the emergent fry stage was less than 0.5%. On 20 August 1979, a random subsample of 500 fish was sorted into 10 groups of 50 fish. These fish were then gradually acclimated over

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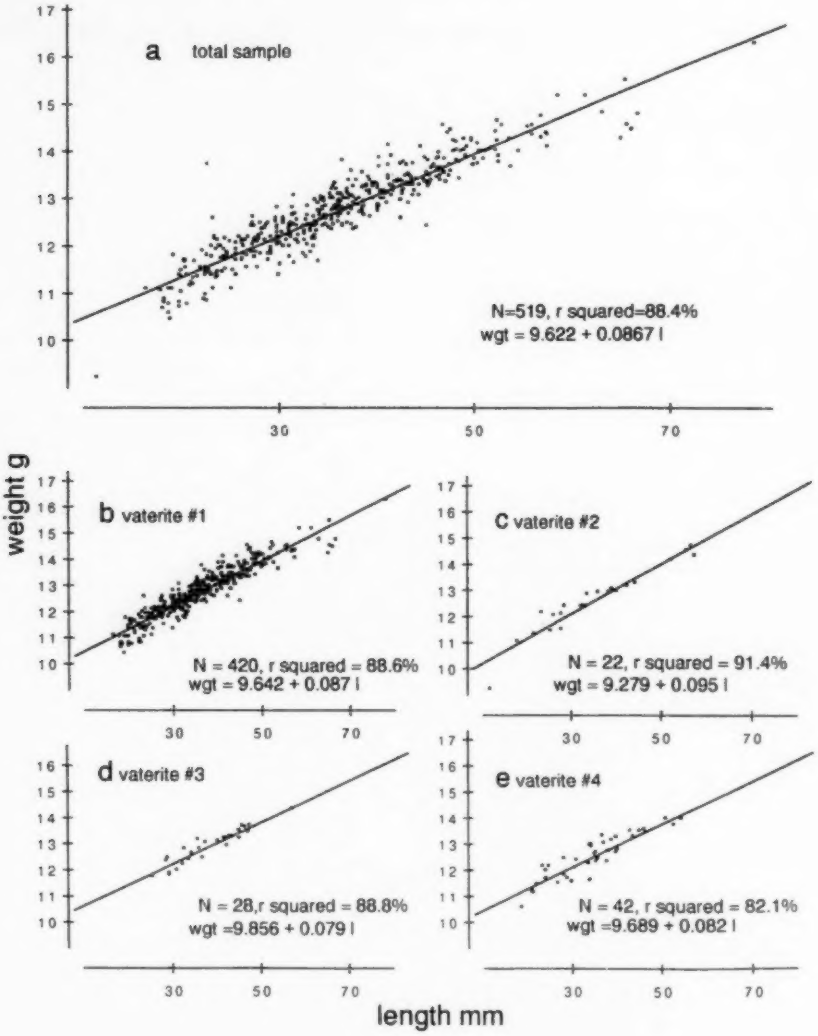
Fig. 1. Fish weights (g) are plotted against fish length (mm). Fitted linear regressions and r^2 values are shown on the graphs (*a*) for the total sample and (*b*–*e*) for fish in each of the vaterite scoring categories 1–4.

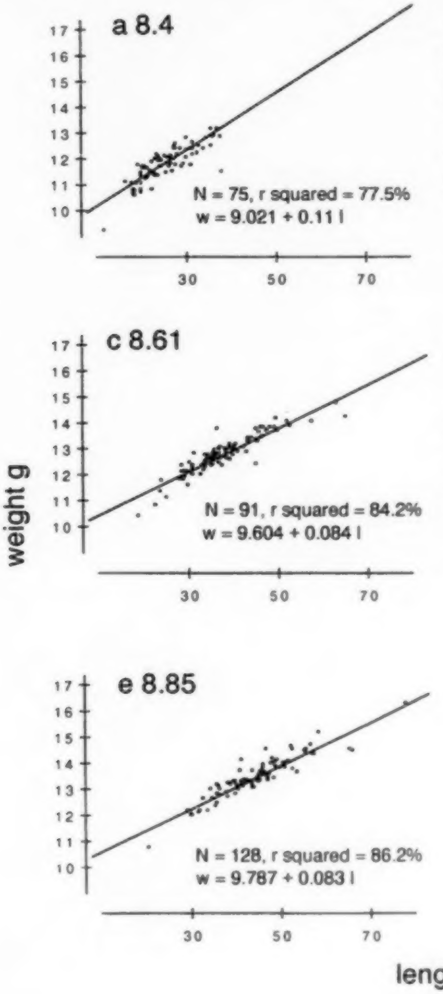


5 days to five different temperature regimes each consisting of two lots of 50 fish in separate tanks. Facilities were provided by the Pacific Biological Station, Nanaimo, B.C. For the duration of the experiment, temperatures were maintained as close as possible to 8, 10, 12, 14, and 16°C. However, the initial acclimation period (of up to 5 days for the highest temperature regimes) leads to a lower mean temperature and increased SE. The consequent mean daily temperatures for each of the five treatment groups over the 43-day experimental period were as follows: 8.31 (control), 7.97 (nominal 8°C), 9.98 (nominal 10°C), 11.70 (nominal 12°C), 13.94 (nominal 14°C), and 15.98°C (nominal 16°C). Records of daily temperatures were kept throughout the experiment. The average temperatures in degrees per day over the entire life of the fish were at the end of the experiment for each temperature treatment group were 8.31 (control), 8.40 (nominal 8°C), 8.52 (nominal 10°C), 8.61 (nominal 12°C), 8.74 (nominal 14°C), and 8.85°C/day (nominal 16°C). Temperature treatment groups are identified by their average values in degrees per day throughout the text. After the 43-day temperature treatment experiment, all of the fish were anaesthetized, weighed, measured, frozen in liquid nitrogen, and stored at –20°C.

During the 43-day temperature experiment 14 fish died in the lowest temperature tanks. Examination of these fish failed to reveal any parasites or infectious agents. At the end of the experiment a further sample of 100 fish was taken randomly from the original population that had been maintained at an average temperature in degrees per day of about 8.31°C. They were anaesthetized, weighed, measured, frozen in liquid nitrogen, and stored at –20°C.

Two morphological types of otoliths were present: an aragonitic

Fig. 2. Fish weight (g) is plotted against fish length (mm) and linear regressions fitted for each temperature treatment group: (*a*) 8.4°C, (*b*) 8.52°C, (*c*) 8.61°C, (*d*) 8.74°C, and (*e*) 8.85°C.



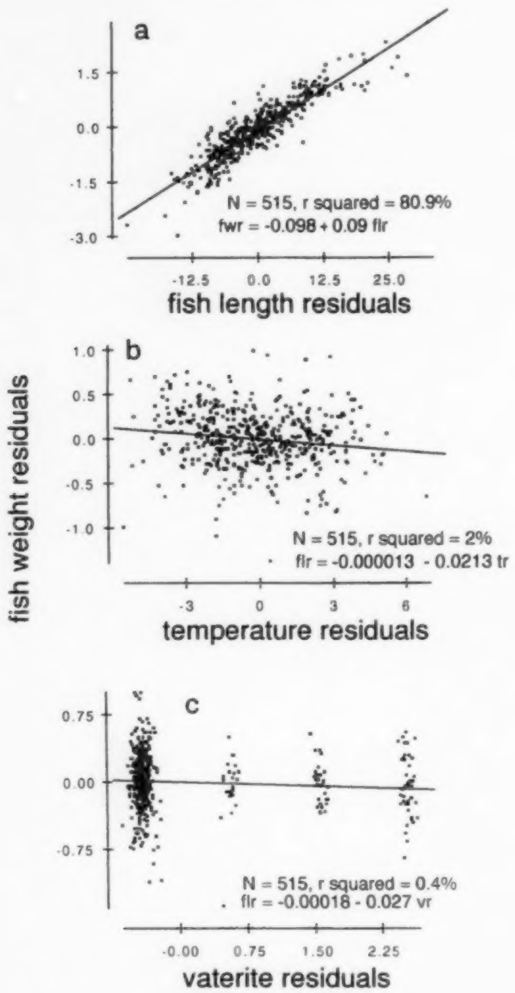
(normal otolith) and a vateritic type. Aragonite and vaterite are two crystalline forms of calcium carbonate that can be distinguished visually. Upon removal, right and left otoliths were kept separate and at that time the fish were sexed. The results reported here are for mixed aragonite and vaterite otoliths. The handedness of vaterite replacement in otoltihs from this sample has been described in Gauldie (1986). Vaterite replacement was scored visually on a scale of 1–4 ranging from complete aragonite to predominantly vaterite following the procedure described in Gauldie (1986). Visual scoring was based on (1) no observable vaterite, (2) traces of vaterite up to one third of total otolith, (3) vaterite content from one third to two thirds of otolith, (4) vaterite content greater than two thirds of otolith. The aragonite data has been reported elsewhere (Gauldie et al. 1986).

All the left otoliths and a random subsample of 128 right otoliths were analyzed by atomic emission spectrometry for the ions iron (Fe), magnesium (Mg), manganese (Mn), zinc (Zn), phosphorus (P), sodium (Na), and strontium (Sr). In addition, the elements cadmium (Cd), copper (Cu), and barium (Ba) were measured, but few (less than 10) otoliths had concentration in excess of the background noise and are not reported. Levels of Fe were also very low, only 41 otoliths had concentratioins higher than background and are not reported.

Each otolith was weighed in a 10-mL acid-washed beaker, then exposed to an oxygen-bearing plasma in a low temperature asher (International Plasma PM-448) to decompose the organic material. The remaining inorganic material was dissolved with 0.05 mL of concentrated nitric acid (Aristar) and diluted by adding 3.5 mL of a solution containing 11.43 mg holmium/L. The final volume was 4.0 mL and the final holmium concentration was 10mg/L. The sample solution was then transferred to an acid-washed polystyrene tube and capped.

The samples were analyzed by inductively coupled argon plasma optical emission spectrometry (Jarrell-Ash 975 Atocomp with spectrum shifter). Standards contained the 10 determined elements Cd, Cu, Fe, Mg, Mn, Zn, Ba, P, Na, Sr, the internal standard holmium, 12.5 mg nitric acid/L, and 1 g Ca/L to match the high calcium background of the sample.

Fig. 3. Fish weight residuals are plotted against (*a*) fish length residuals, (*b*) temperature residuals, and (*c*) vaterite residuals. Linear regressions are fitted for each plot.



The usual lowest quantity determinable with this technique is five times the standard deviation of blank (Butler et al. 1975), but the viscosity of the samples was high and variable since they had calcium concentrations ranging from 800 to 1200 mg/L. To overcome anomalies generated by flow rate differences due to different viscosities, the lowest quantity determinable was set at 10 times the standard deviation of blanks. Consequently, different elements had different lowest determinable quantities that results in differences in sample size from element to element.

Statistical analyses (and statistical nomenclature) were derived from the Data Desk statistical software package (Data Descriptions Inc., P.O. Box 4555, Ithaca, NY 14852). This study is concerned with interactions between metal ions in the otolith and other metal ions in the otolith, as well as temperature, otolith weight, fish weight, and fish length. In the absence of physiological models of such interactions, the most useful question that can be answered from statistical methods is how much of the variation observed in a metal ion can be explained by the variation in other factors? One way to answer these questions is to produce a multiple linear regression leading to a table of partial regression parameters (constant, slope, probability that the slope is significantly different from zero, etc.). None of this information is particularly useful in asking the basic question of how much variation is linked between metal ions, nor does such information cast any light on the differences in metal ion distribution among individuals. However, multiple linear regression automatically provides partial linear regressions between the variables involved. Generally, a partial linear regression allows one to assess the effects of one variable on another when the effects of all other interactions are removed. Partial linear regressions are a more heuristic approach to the problems of interactions than multiple regression tables, and are used for that purpose in this study. The square of the regression coefficient, r^2, provides a measure of how much of the variation in one variable is explained by the variation in another. In the case of partial regressions, the r^2 is degraded as a measure of correlation because of the difficulty in exactly calculating covariances and variances of two variables extracted statistically from many variables. However, the r^2 value of the partial regressions does allow the ranking of interactions between variables from higher to lower correlations that provides a qualitative assessment of the variation contributed by any one covariable to another covariable from a group of simultaneous measurements. The partial linear regression graphs also show the different distributions of variables among individuals and are used for ranking in this study.

Results

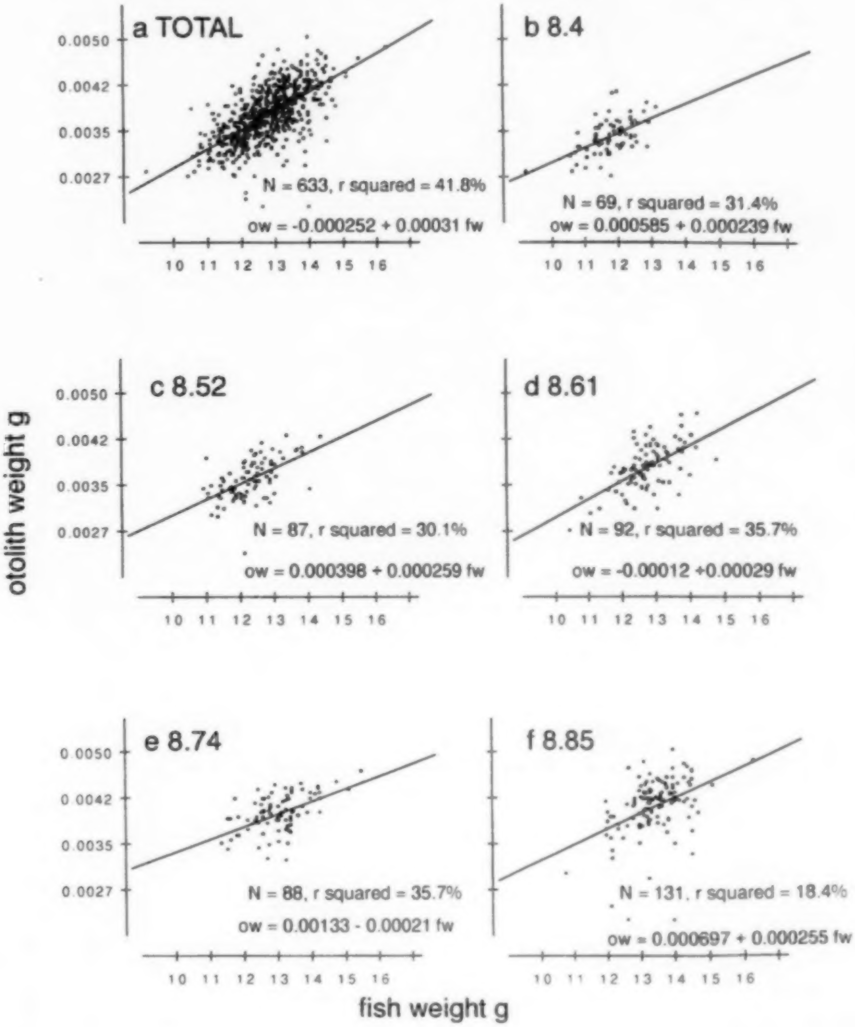
One of the most striking features of otolith chemistry data is the variation between individual fish. In this experiment in which conditions were kept as uniform as possible the high-level individual variation is an important observation. Samples of left and right otoliths were processed from 128 fish randomly subsampled. There were no statistically significant differences between left and right otoliths in the categories: otolith weight, degree of vaterite substitution, and parts per million concentrations of the elements Sr, Na, P, Mg, Mn, Zn, and Fe. Sex determination was possible only in larger fish, but for the 91 females and 121 males that it was possible to sex, there were no statistically significant differences between males and females in the following categories: otolith weight, fish weight, fish length, degree of vaterite replacement, and parts per million concentrations of the elements Sr, Na, P, Mg, Mn, Zn, and Fe. The fish separated in the final experiment into two lots of 50 each for each of the experimental temperture regimes showed no statistically significant differences in any of the measured categories.

Contingency tables were used to examine possible interactions in the categories: (1) handedness and vaterite replacement and (2) sex and vaterite replacement. There were no statistically significant differences between observed and expected frequencies.

Fish weight and length relationships

The slopes of separate regressions for fish weight to fish length relations corresponding to the whole sample and to vaterite scores 1–4 (Figs. 1*a*–1*e*) were similar. By using an arbitrary sliding transformation of fish weight (w) and fish length (l), $w = l^{3.3}$, it was possible to increase the fit of the regression slightly, from $r^2 = 88.4$ to 89.1% in the total sample, but there was less than 2% change in r^2 in the smaller subsamples. Consequently, simple linear regressions were used throughout.

Fig. 4. Otolith weights are plotted against fish weights for the total sample (*a*) and otolith weights are plotted against fish weights for each temperature treatment group with fitted linear regressions: (*b*) 8.4°C, (*c*) 8.52°C, (*d*) 8.61°C, (*e*) 8.74°C, and (*f*) 8.85°C.



Separate regressions for fish weight to fish length corresponding to each temperature treatment groups (Figs. 2*a*–2*e*) had different slopes, but these were clumped in such a way that the slopes of temperature groups 8.61 and 8.85° were similar and temperature groups 8.52° and 8.74° were similar; however, both groups differed from each other and from the 8.4° temperature group. The steepest slope, i.e., the greatest change of fish length with fish weight, was in the lowest temperature group.

An alternative way to examine relationships between fish weight and length is to use partial regression statistics. Notwithstanding the problem of assigning an exact interpretation to r^2 values of plots of residuals, the r^2 of the residual plots from partial regressions imply that a large proportion (r^2 = 80.9%) of the variation in fish weight residuals was explained by the variation in fish length residuals (Fig. 3*a*), but smaller proportions of the variation in fish weight were explained by the variation in temperature residuals (r^2 = 2%; Fig. 3*b*) and vaterite replacement residuals (r^2 = 0.4%; Fig. 3*c*). The partial regression statistics summarized in Figs. 3*a* and 3*b* show that compared with fish length, temperature, and vaterite replacement have a much weaker relationship to fish weight.

Otolith weight

Plots of regressions imply that less than half of the variation in otolith weight (r^2 = 41.8%; Fig. 4*a*) is explained by the variation in fish weight in the total sample. Regressions of otolith weight on fish weight for each temperature treatment

Table 1. Multiple regression of Sr on nine independant variables (total r^2 = 89.4%).

(A) Regression.

Source	SS	df	MS	F
Regression	95 920 008	9	1 065 779	161
Residual	1 075 190	162	6636.97	

(B) Coefficients.

Variable	Coefficient	SE of coefficient	t	p
Constant	817.281	129.1	6.33	≤0.0001
Temperature	7.18019	2.647	2.71	0.0074
Fish weight	8.62481	9.447	0.913	0.3626
Vaterite	−146.472	11.87	−12.3	≤0.0001
Otolith weight	−21.7370	22.18	−0.980	0.3284
Mg	−0.179 667	0.0843	−2.13	0.0346
Mn	0.153 083	1.459	0.105	0.9166
Zn	−0.156 832	0.2865	−0.547	0.5848
P	−0.011 524	0.0366	−0.315	0.7530
Na	0.041 378	0.0078	5.32	≤0.0001

Table 2. Multiple regression of Sr on four principal independant variables (total r^2 = 88.7%).

(A) Regression.

Source	SS	df	MS	F
Regression	18 863 677	4	4 715 919	698
Residual	2 371 500	351	6756.41	

(B) Coefficients.

Variable	Coefficient	SE of coefficient	t	p
Constant	861.326	35.81	24.1	≤0.0001
Temperature	5.270 07	1.509	3.49	0.0005
Vaterite	−133.410	8.565	−15.6	≤0.0001
Mg	−0.290 143	0.0615	−4.71	≤0.0001
Na	0.036 196	0.0052	7.02	≤0.0001

group are shown in Figs. 4*b*–4*f*. The slopes of otolith weight on fish weight were statistically heterogeneous among all of the temperature treatment groups except the 8.4 and 8.52°C groups.

Plots of otolith weight residual regressions on fish weight residuals, temperature treatment residuals, and vaterite score residuals are shown in Figs. 5*a*–5*c*. Partial regression statistics imply that vaterite replacement accounts for a small part of the variation (r^2 = 2.5%) in otolith weight. The strongest effect on the variation in otolith weight is fish weight (r^2 = 27%). Surprisingly, the r^2 value of the regression of temperature variation on the variation in otolith weight is 16.6%, in contrast to the small amount of variation in fish weight (r^2 = 2%; Fig. 3*b*) explained by the variation in temperature.

Otolith chemistry

The degree of vaterite replacement in the otoliths had a strong effect on the chemistry of the otolith. For example, regression of otolith Sr against otolith Mg showed a decrease in Sr with increasing Mg that is evidently proportional to the degree of vaterite replacement (Fig. 6).

The multiple regression of Sr on the variables temperature, fish weight, otolith weight, vaterite, Mg, Mn, Zn, P, and Na is shown in Table 1. The regression shows that all of the variables together can explain 89.4% of the variation in Sr. However, examination of Table 1 shows that only four variables have partial regressions whose slopes are significantly greater than zero (i.e., α = 0.05): temperature, vaterite, Mg, and Na. A second Sr regression (Table 2) using only temperature, vaterite, Mg, and Na as independant variables showed a small reduction in r^2 from 89.4 to 88.7%. Inspection of the *t*-ratios and probabilitites shows that the order of influence on Sr variation was vaterite, Na, Mg, and temperture. These statistical effects can be made more intuitively obvious by plotting

Table 3. Multiple regression of Mg on nine independant variables (total r^2 = 7.26%).

(A) Regression.

Source	SS	df	MS	F
Regression	3 707 315	9	411 924	73.5
Residual	907 880	162	5604.20	

(B) Coefficients.

Variable	Coefficient	SE of coefficient	t	p
Constant	−34.9542	132.5	−0.264	0.7923
Temperature	4.328 36	2.463	1.76	0.0808
Fish weight	1.496 79	8.702	0.172	0.8637
Vaterite	80.8637	13.80	5.86	≤0.0001
Otolith weight	1.903 57	20.44	0.093	0.9259
Sr	−0.151 709	0.0712	−2.13	0.0346
Mn	0.836 773	1.339	0.625	0.5330
Zn	0.536 520	0.2601	2.06	0.0407
Na	$-5.683\ 88 \times 10^{-3}$	0.0077	−0.735	0.4636
P	0.0809 72	0.0330	2.45	0.0152

Table 4. Multiple regression of Mn on nine independant variables (total r^2 = 15.5%).

(A) Regression.

Source	SS	df	MS	F
Regression	774.972	9	86.1080	4.48
Residual	3116.78	162	19.2394	

(B) Coefficients.

Variable	Coefficient	SE of coefficient	t	p
Constant	−13.5555	7.692	−1.76	0.0799
Temperature	−0.187 817	0.1450	−1.30	0.1969
Fish weight	0.624 369	0.5076	1.23	0.2204
Vaterite	0.848 949	0.8875	0.957	0.3402
Otolith weight	1.425 54	1.192	1.20	0.2336
Sr	$4.437\ 61 \times 10^{-4}$	0.0046	0.625	0.5330
Mg	$2.872\ 67 \times 10^{-3}$	0.0046	0.625	0.5330
Zn	0.0389 03	0.0151	2.57	0.0111
P	$4.019\ 64 \times 10^{-3}$	0.0019	2.07	0.0402
Na	$-1.042\ 76 \times 10^{-5}$	0.0005	−0.023	0.9817

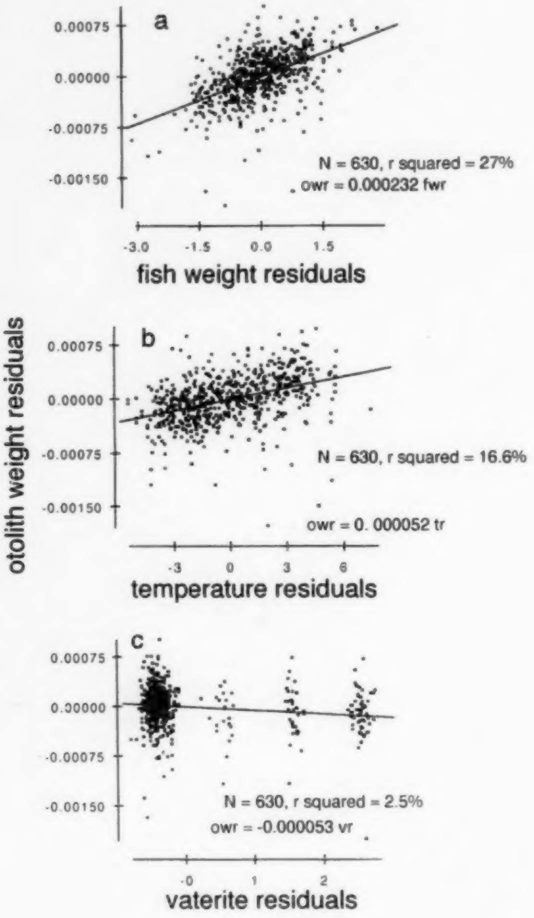
strontium residuals against temperature, vaterite, Mg, and Na residuals respectively (Figs. 7*a*–7*d*).

Similar partial regression analyses were carried out for Mg, Mn, Zn, P, and Na (Tables 3–7). The element Mg shares a strong correlation with vaterite as does Sr. Inspection of Table 3 shows significant regression slopes ($\alpha \le 0.05$) for Mg on vaterite, Sr, Zn and P, but the *t*-ratios are low reflecting low partial regression r^2 values (<10%) in plots of residuals. The partial regression of Mn (Table 5) shows significant regression slopes on Zn and P, but low *t*-ratios imply weak statistical relationships. The element Zn shows significant regression slopes on Mg and P, but low *t*-ratios imply low r^2 values. Phosphorous (Table 7) shows significant regression slope on temperature, vaterite, Mg, Mn, and Na. However, *t*-ratios were low, implying low partial regression r^2 values. The partial regression of Na residuals on Sr residuals had on r^2 value of 14.4%.

Discussion

It would be very difficult to devise an experiment in which one or more of the elements measured in the otolith was varied

Fig. 5. Otolith weight residuals are plotted against (*a*) fish weight residuals, (*b*) temperature residuals, and (*c*) vaterite residuals. Linear regressions are fitted to all plots.



while the others were held constant. Consequently, most otolith chemistry data represent measures of the sum of different interactions among elements that are occuring simultaneously in the precipitation environment of the endolymph, as well as the measured values of the elements by themselves. Sorting out interelement variances by analysis of variance or principal components return results that are difficult to interpret chemically or biologically and cannot be readily extrapolated to other data sets. Multiple regression (which is statistically equivalent to principal component analysis) provides a more heuristic approach from which partial regression plots allow the viewer to visualize the interaction taking place. Although the r^2 values of partial regressions may not be exactly quantitative measures of the normalized covariance among pairs of elements, they do offer a qualitative ranking of the degree of influence of one element on another. That is the sense in which the r^2 values of partial regressions have been used in this study. More detailed discussions of partial regression statistics are in Chatterjee and Price (1977) and Cook and Weisberg (1982).

One of the striking features of the metal ion data from the otoliths of this group of *O. tshawytscha* was the large variation among individual otoliths. The observed variation was unlikely to be due to experimental error because of the similarity in mean values of measured variables between left and right otoliths. The ratio of mean to range of values for the measured elements were as follows: Na (0.58), P (0.4), Zn (0.08), Mn (0.5), Mg (0.23), and Sr (0.75). There are three possible mechanisms that could cause the high individual variation observed in otolith chemistry.

(1) Chemicals in the surrounding water pass into the fish and then into the otolith either directly as soluble ions or as ions dissolved from food.

(2) Chemicals in the body fluid pass out of the blood or perilymph into the endolymph, so that concentrations of those chemicals in the blood or body fluid are tracked by their concentrations in the endolymph, which in turn is recorded directly in the chemistry of the otolith.

(3) Chemicals in the endolymph enter the growing otolith crystal as a function of the physical chemistry of the solid–water interface of the otolith crystal independently of concentrations in either the environment or body fluids.

The first mechanism of surrounding chemicals could not be tested in this experiment because both the surrounding water and food supplied were standardized for all fish.

The second mechanism of body fluid chemicals implies that physiological changes, such as growth, cause variation in the transported ion concentrations in the body fluids (e.g., calcitonin-bound calcium), that lead to changes in the relative concentrations of chemicals in the otolith. This mechanism is difficult to test because of the difficulties in measuring the ATP-driven processes that release protein-transported ions and facilitate their passage across cell and membrane boundaries into the endolymph. Temperature treatment groups showed significant differences in body size and otolith size. Because all fish were the same age, differences in body weight and otolith weight are proxies for body growth rate and otolith growth rate, respectively, that allow the general question of the effects of growth on otolith chemistry to be tested.

A temperature change from 8 to 16°C for 43 days caused a 30% change in fish size even though 43 days represents only 12.3% of the total life of the fish. The differences in fish sizes within temperature treatment groups smoothly integrated into a general relationship between weight and length for the entire sample. Otolith weight increased with increasing fish weight, but the relationship between otolith weight and fish weight was statistically much weaker than the relationship between length and fish weight. Otolith weight was effectively decoupled from fish weight in the sense that only 27% of the variation in otolith weight was explained by the variation in somatic weight. Very little of the variation in otolith chemistry was explained by the variation in otolith weight and fish weight either singly or in concert. Therefore, the somatic and otolith weight changes caused by temperature in this group of *O. tshawytscha* provided a limited test of the

Table 5. Multiple regression of Zn on nine independant variables (total r^2 = 24.2%).

(A) Regression.

Source	SS	df	MS	F
Regression	31 662.6	9	3518.06	7.06
Residual	80 712.4	162	498.225	

(B) Coefficients.

Variable	Coefficient	SE of coefficient	t	p
Constant	155.723	37.57	4.14	≤0.0001
Temperature	1.019 80	0.7371	1.38	0.1684
Fish weight	–0.256 363	2.595	–0.099	0.9214
Vaterite	–1.174 51	4.528	–0.259	0.7957
Otolith weight	–7.930 09	6.062	–1.31	0.1927
Sr	–0.001 177 3	0.0215	–0.547	0.5848
Mg	0.047 698	0.0231	2.06	0.0407
Mn	1.007 43	0.3919	2.57	0.0111
P	$-8.200\ 04 \times 10^{-3}$	0.0100	–0.820	0.4134
Na	$-1.269\ 67 \times 10^{-3}$	0.0023	–0.550	0.5831

Table 6. Multiple regression of P on nine independant variables (total r^2 = 21.3%).

(A) Regression.

Source	SS	df	MS	F
Regression	1 693 540	9	188 171	6.14
Residual	4 961 591	162	30 627.1	

(B) Coefficients.

Variable	Coefficient	SE of coefficient	t	p
Constant	1225.78	294.5	4.16	≤0.0001
Temperature	–21.9464	5.552	–3.95	0.00001
Fish weight	–14.9296	20.31	–0.735	0.4634
Vaterite	–114.114	34.36	–3.32	0.0011
Otolith weight	40.4755	47.67	0.849	0.3971
Sr	–0.053 179	0.1687	–0.315	0.7530
Mg	0.442 513	0.1803	2.45	0.0152
Mn	6.398 84	3.094	2.07	0.0402
Zn	–0.504 077	0.6147	–0.820	0.4134
Na	–0.041 985	0.0178	–2.36	0.0196

body fluid chemicals mechanism and showed that the body fluid chemicals mechanism was not significant.

The third mechanism of solid–water interface chemistry can be tested by the interactions between the carbonate chemistry of the otolith and the chemicals Sr, Mg, Mn, P, Na, Fe, and Zn.

The naturally occurring replacement of aragonite by vaterite provides a probe to examine the effect of crystal structure on the chemistry of the otolith. A general theoretical treatment of the chemistry of the solid–water interface in crystal growth and dissolution is provided by Stumm (1992). Vaterite replacement of aragonite had a strong effect on the chemistry of the otolith. The effect of vateritic was greatest on Sr and Mg with a lesser effect on P, Na, Mn, and Zn. All of the otoliths scored as vaterite were mixtures of vaterite and aragonite. For these otoliths, the vaterite and aragonite components that were growing simultaneously in the endolymph have reacted differently to the same concentrations of chemicals in the same endolymph at the same time. The vaterite effect showed that changes in the physical chemistry of the otolith alone, independent of the chemistry of the endolymph, resulted in large changes in otolith chemistry.

The variation in Sr in otoliths in this experiment was strongly influenced by vaterite replacement. However, when

Table 7. Multiple regression of Na on nine independant variables (total r^2 = 55.8%).

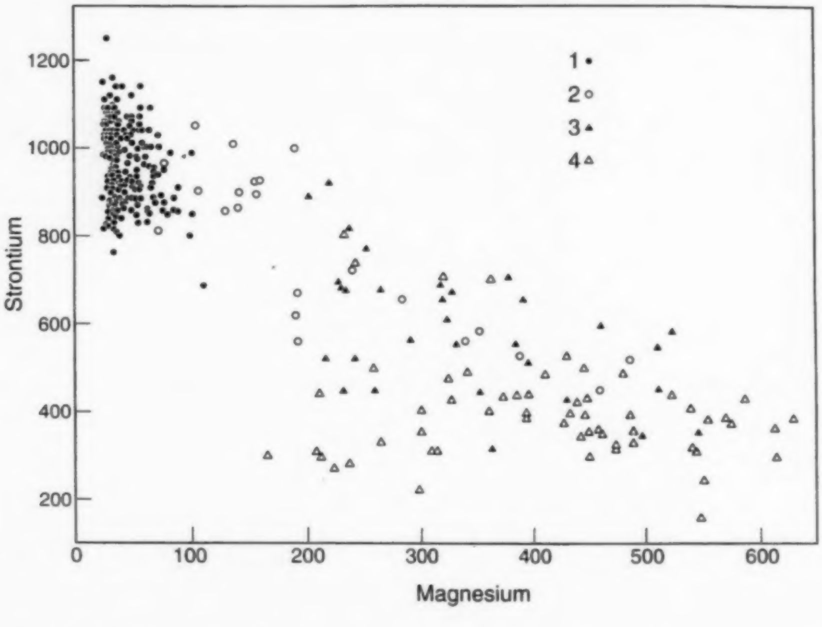
(A) Regression.

Source	SS	df	MS	*F*
Regression	12 755 710	9	14 417 301	25.0
Residual	93 302 624	162	575 942	

(B) Coefficients.

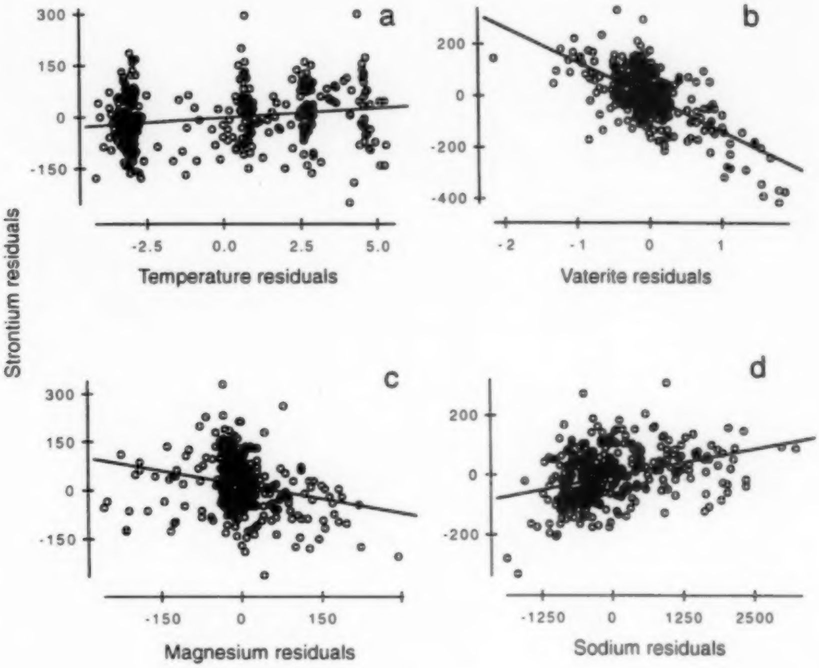
Variable	Coefficient	SE of coefficient	*t*	*p*
Constant	3757.70	1311	2.87	0.0047
Temperature	−43.2623	24.98	−1.73	0.0852
Fish weight	−109.360	87.81	−1.25	0.2148
Vaterite	130.864	153.6	0.852	0.3956
Otolith weight	83.0882	207.1	0.401	0.6888
Sr	3.590 71	0.6753	5.32	≤0.0001
Mg	−0.584 132	0.7952	−0.735	0.4636
Mn	−0.312 157	13.59	−0.023	0.9817
Zn	−1.467 72	2.669	−0.550	0.5831
P	−0.789 527	0.3350	−2.36	0.0196

Fig. 6. Strontium concentration (ppm) is plotted against magnesium concentration (ppm) for all of the otoliths. The vaterite scores 1–4 show a progressive increase in the relative amount of magnesium to strontium with the progressive increase in vaterite replacement.



the vaterite effect was statistically accounted for, there were significant correlations between Sr variation and variation in temperature and otolith weight (which is an analog of otolith growth rate as all fish were of the same age). These correlations show that observations of Sr dependance on otolith growth rate and ambient temperature are not mutually exclusive. The relation between Sr and Na is difficult to explain either as changes in endolymph Na or similarities in Sr and Na carbonate chemistry. It is probable that there is another variable, or group of variables, as yet not measured (e.g., pH) that may control both Sr and Na deposition leading to a spurious correlation. The chief problem in using the correlation between Sr and otolith weight and ambient temperature in any practical application lies in overcoming the noise introduced

Fig. 7. Strontium residuals are plotted with fitted linear regressions against (*a*) temperature residuals, (*b*) vaterite residuals, (*c*) Mg residuals, and (*d*) Na residuals.



by the high level of variation between individuals. This experiment shows differences in chemistry between individual otoliths from fish reared under nearly identical conditions can be as great as those between fish caught in the wild.

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Genetic impact on two wild brown trout (*Salmo trutta*) populations after release of non-indigenous hatchery spawners

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Abstract: A genetically marked hatchery strain of brown trout (*Salmo trutta*) was employed to study the genetic impact from non-indigenous hatchery fish on wild stocks. The hatchery spawners were released in autumn 1989 into the spawning localities of two wild trout stocks in River Øyreselv, Norway. The F_1 generation was sampled and genotyped at the 0+, 1+, and 2+ stages. Juveniles carrying the genetic markers were found in both localities, proving that the introduced spawners had spawned among themselves and with the wild stocks. The genetic contribution from the hatchery fish was estimated at 19.2 and 16.3% at the 0+ stage in the two wild stocks. Estimates of survival rates of 0+ trout revealed that survival was nearly three times higher in wild trout than in hybrids of wild and introduced trout, possibly because of a difference between introduced and wild stocks in size of eggs and alevins. The frequency of the marker alleles in the F_1 generation declined during the 2-year observation period.

Résumé : Nous avons utilisé une lignée d'élevage génétiquement marquée de truite brune (*Salmo trutta*) pour étudier l'impact génétique des poissons d'élevage non indigènes sur les stocks sauvages. À l'automne 1989, nous avons relâché des gniteurs élevés en pisciculture dans les frayères de deux stocks de truite sauvages de la rivière Øyreselv, en Norvège. Nous avons prélev un échantillon de la génération F_1 et en avons établi les génotypes aux stades 0+, 1+ et 2+. Nous avons retrouvé dans les deux localités des juvéniles portant les marqueurs génétiques, ce qui prouve que les géniteurs introduits se sont croisés entre eux et avec les stocks sauvages. Les contributions génétiques des sujets d'élevage du stade 0+ des deux localités ont été estimées à 19,2 et à 16,3%. Chez les truites du stade 0+, le taux estimatif de survie était trois fois plus élevé pour les sujets sauvages que pour les hybrides entre sujets sauvage et introduit, sans doute à cause d'une différence entre les stocks sauvages et introduits quant à la taille des oeufs et des alevins. La fréquence des allèles marqueurs chez la génération F_1 a diminué au cours des deux années d'observation.

[Traduit par la Rédaction]

Introduction

To what extent do fish that escape or are released from hatcheries and fish farms affect the genetic characteristics and ecology of wild populations? This is an important question in the discussion of possible impacts of escaped fish on wild stocks in Norway as well as in many other countries. In Norway, the discussion has intensified as the production of farmed Atlantic salmon (*Salmo salar*) has expanded, with a corresponding increase in the number of escaped farmed salmon in coastal areas and rivers. At the same time the number of harvested wild salmon in many rivers has been showing a downward trend. The production of farmed salmon in Norway alone has increased from 4 000 t in 1980 to about 260 000 t in 1995, while the annual yield of wild salmon has varied between 1000 and 2500 t during the last 30 years according to Norwegian fishery statistics. The number of wild salmon spawners in Norwegian rivers has been estimated at 100 000 individuals (Ståhl and Hindar 1988), while the official estimate of the number of escaped salmon in 1989 was 2 million. The number of hatchery smolts produced exceeds 90 million, while the number of wild salmon smolts leaving Norwegian rivers has been estimated at approximately 6 million. Thus, the number of cultivated salmon is high compared with the number of wild salmon. However, the genetic impact is not dependent on numerical relationships alone. The degree of impact also depends on the extent of genetic adaptation of wild populations, the degree of genetic differentiation between wild and cultivated populations, and on the performance of escaped cultivated individuals (Fleming and Gross 1993).

There is little empirical data on genetic adaptation at the population level and on genetic impact by various categories of cultivated populations on wild stocks of salmonids. For this reason, discussions of genetic impact from aquaculture, ocean ranching, and enhancement programmes often rely on theoretical considerations. Genetic marking has been recommended as a valuable tool in studies on reproductive success of released and escaped fish and their impact on wild stocks (Allendorf and Utter 1979; Shaklee 1983; Taggart and Ferguson 1984; 1986; Skaala and Jørstad 1994).

The purpose of our experiment was to study the short term impact by non-indigenous hatchery brown trout (*Salmo trutta)* on genetic characteristics and population dynamics of indigenous stocks in a natural habitat.

The brown trout was chosen as a model species in this experiment for several reasons. Firstly, it is hypothesized

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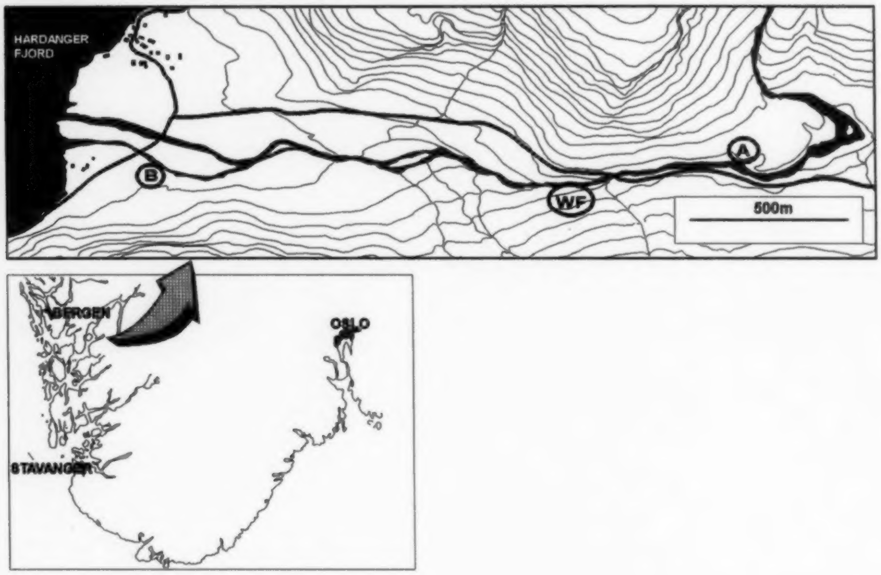
Fig. 1. Map of southern Norway with the field locality indicated: enlarged map of River Øyreselv showing above (A) and below (B) waterfall (WF) sections.



that adverse effects of population mixing would be most pronounced and most readily detected in species where a large portion of the total genetic variability is divided among populations. The brown trout is one of the most polymorphic vertebrate species known, with reproductive barriers occurring between populations separated by small distances, and with a wide variation in life-history characters (Ryman 1983; Ferguson 1989). Secondly, suitable genetic markers are abundant in brown trout as opposed to Atlantic salmon. Finally, as brown trout are smaller than Atlantic salmon, and inhabit smaller rivers and creeks, there are also practical advantages for experimental design in using this species as a model.

Materials and methods

The area

The River Øyreselv drains into the Hardangerfjord on the west coast of Norway (Fig. 1). The river has a population of Atlantic salmon, but anadromous and resident brown trout is the dominant species by numbers. A waterfall impassable to anadromous fish is located about 1.5 km from the fjord. The lower part of the river is divided in two parallel branches, each 375 m long. Two sections of the river were chosen for the experiment; section A above the waterfall, and section B, the smallest side branch below the waterfall, where the discharge is generally less than 1–2 $m^3 \cdot s^{-1}$. Higher discharges usually occur during snowmelt and during late autumn. Resident and anadromous brown trout spawn in section A and B, respectively. In section A, a large part of the area is suitable for spawning, while in section B the river bed is dominated by large stones and boulders, with small areas of suitable spawning substrate. The average width of the river channel in section B is 10.0 ± 2.9 m (mean ± SD). During our sampling, the temperature ranged from 0–2.5°C in winter to 8.5–12.5°C in autumn.

Genetically marked non-indigenous hatchery spawners were released in both sections in autumn 1989. The released spawners were all first-generation hatchery fish grown under standard rearing conditions in fibreglas tanks and fed only commercial dry feed. Thus, they had no previous experience with natural river environments, and limited physical conditioning compared with wild spawners. In section A, video observations of the spawning behaviour of hatchery and wild trout were made (Skaala et al. 1993). In section B, a mark–recapture study on juveniles was carried out to estimate the number and survival rate of offspring resulting from wild and introduced trout, and from combinations of these, and to assess changes in population parameters due to the potential genetic impact. Thus, the major effort was concentrated in section B.

Genetic markers and electrophoresis

In 1986, production of a genetically marked trout strain was initiated by selecting and mating individuals with specific phenotypes (Skaala and Jørstad 1988). During 1988, genetic information on a number of wild trout populations was recorded to find a suitable locality for the experiment, and electrophoretic studies were conducted. These included the following 12 enzyme systems: aspartate aminotransferase (AAT, E.C. 2.6.1.1), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), adenylate kinase (AK, E.C. 2.7.4.3), creatine kinase (CK, E.C. 2.7.3.2), esterase (EST, E.C. 3.1.1.-), glycerol-3-phosphate dehydrogenase (G3PDH, E.C. 1.1.1.8), isocitrate dehydrogenase (IDHP, E.C. 1.1.1.42), lactate dehydrogenase (LDH, E.C. 1.1.1.27), malate dehydrogenase (MDH, E.C. 1.1.1.37), malic enzyme (MEP, E.C. 1.1.1.40), glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9), and phosphoglucomutase (PGM, E.C. 5.4.2.2), putatively encoded by 32 loci. Allelic frequencies were calculated according to Ferguson (1980). Details of the electrophoretic methods are given by Skaala and Jørstad (1987). Two types of genetic markers were used in each section, a visible marker (*PIGM**) and an isozyme marker (Table 1). The visible marker includes a genetic polymorphism with an allele (f) causing a fine spotting pattern on the body and fins that can be easily distinguished from the large spots commonly found in brown trout (Skaala and Jørstad 1987, 1988). Juveniles homozygous for this variant allele lack parr marks. Heterozygous individuals are intermediate between the fine-spotted

Table 1. Allele frequencies in the marker loci (*LDH-5**, *MDH-2**, and *PIGM**) of the introduced fine-spotted trout strain, of the wild trout in sections A and B in River Øyreselv, and in neighbouring wild populations.

Population	*N*	*LDH-5** *100*	*MDH-2** *152*	*PIGM** (*f*)
Hatchery	50	0.730	0.800	1.000
River Øyre A	73	0.760	0.021	0.000
River Øyre B	103	0.034	0.272	0.000
River Enes	73	0.036	0.224	0.000
River Fosså	71	0.014	0.120	0.000
Lake Opsanger	83	0.059	0.524	0.000
Lake Gygrastol	96	0.000	0.240	0.000

and common genotypes in appearence, and juveniles have irregular and disrupted parr marks (Fig. 2). The two isozyme markers used were *MDH-2*152* (section A) and *LDH-5*100* (Section B). Samples of the 1990 year-class were collected in 1990 (0+), 1991 (1+), and 1992 (2+) to record genotypic distributions and calculate allelic frequencies. The genetic contribution made by the introduced stock was estimated by means of allelic frequency proportionality (Taggart and Ferguson 1986) as:

$$\left(\frac{P_{\mathrm{n}} - P_{\mathrm{m}}}{P_{\mathrm{n}} - P_{\mathrm{f}}}\right) \times 100$$

where P_{n} is the allelic frequency in the native population, P_{m} in the mixed population, and P_{f} is the allelic frequency in the introduced hatchery population. Genotypic distributions in marker loci were tested for heterogeneity by using a *G*-test (Sokal and Rohlf 1969), and differences in allelic frequencies were tested by the BIOSYS-1 PC program package (Swofford and Selander 1981).

Abundance of trout

To address the potential ecological implications of induced gene flow, in terms of changes in production (Crozier 1993), population dynamic studies were incorporated in the experiment in section B. The number of wild spawners in section A was estimated by diving surveys and fish counting, and a sample of adult fish was collected by gillnetting to record the lengths and weights of spawners. The 0+ parr were sampled by divers and by electrofishing. In section B the number of spawners was recorded by electrofishing during the spawning seasons. Based on population estimates and genetic marking, the numbers in each year-class and the survival of offspring resulting from wild and reared fish were assessed. In section B, the number of 0+, 1+, and older parr were estimated by a mark–recapture method ((Chapman's adjusted Peterson method) (Ricker 1975) in August 1989, March and August 1990, February and August 1991, March and September 1992, and February 1993. Confidence limits for the estimates were obtained from Ricker (1975, appendix II), using the number of recaptures of marked fish as the entering variable.

The fish were captured by an electric shocker, anaesthetized with benzocaine, measured (total length in millimetres) and marked either with alcian blue dye at the base of the pelvic, ventral, anal, and caudal fins, using a Jet inoculator (Hart and Pitcher 1969; Pitcher and Kennedy 1977) or by removing the adipose fin. All samplings, except in August 1990, were carried out at low and approximately similar stream flows, in which the whole stream bed could be waded. In August 1990, the first sampling was carried out during a flood, and had to be restricted to a narrow zone along the banks of the stream, resulting in a low capture probability for 1+ and older parr.

In August 1989, the 0+ parr were not marked. Thus, no Petersen estimate was obtained. Likewise, no Peterson estimates of 0+ parr were obtained in March 1990 and February 1991 because of the low number of recaptures. For these dates, the number of 0+ was estimated by catch per unit effort (CPUE), according to the equation $C = qN$, where C is the number caught during the first sampling run, q is the catchability, and N is the number of parr present. For the other months, estimates of numbers of 0+ were obtained both by mark–recapture and CPUE. The catchability of 0+ parr was estimated according to the relationship $q = aL^b$, where L is the mean length in millimeters and a and b are constants. The length-classes used in these calculations were obtained from Borgstrøm and Skaala (1993), and q for each cohort of 0+ was estimated separately for each sampling month Table 2). Survival (*S*) estimates of 0+ from August–September to February–March have been obtained by using the estimated numbers from the CPUE method ($S = N_1/N_2$, where N_1 and N_2 are numbers of parr in August–September and February–March, respectively) and by the use of marked fish, as described by Ricker (1975) ($S = R_{12}\ M_2/M_1\ (R_{22} + 1)$, where M_1 and M_2 are number of marked fish in August–September and February–March, respectively, and R_{12} and R_{22} are the numbers of recaptures of the two markings after the second marking).

Release of non-indigenous hatchery spawners

In autumn 1989, spawners homozygous for the fine-spot allele were released in spawning areas of wild brown trout in River Øyre. The numbers of reared and genetically marked trout released in section A were 56 (43 males and 13 females), and 104 (67 males and 37 females) in section B. The individual length of the released spawners varied between 25.5 and 42.5 cm in section A, and between 32.0 and 42.5 cm in section B, corresponding to masses between 0.2 and 1.0 kg. Each spawner was tagged individually with a Floy anchor tag. Diving surveys and video recordings were made during late autumn and winter to estimate the number of released trout still present at the spawning grounds.

Results

Genetic impact

All samples of wild trout from year-classes hatched before the release experiment matched the expected Hardy–Weinberg distribution. The visible marker (fine-spotted) was not found in wild trout in the River Øyreselv or in the nearby rivers. The *MDH-2*152* allele common in the marked strain ($p = 0.800$) was rare in the population in section A ($p = 0.02$). The *LDH-5*100* allele, present at a high frequency ($p = 0.730$) in the marked strain, was present at a low frequency ($p = 0.03$) in the anadromous brown trout in section B in River Øyreselv as well as in the neighbouring rivers (Table 1). Thus, two independent genetic markers were available in each section, one visible and one allozyme.

In forty-three 0+ parr sampled in section A above the falls in October 1990, two individuals homozygous for the visible marker, and seven heterozygous individuals were identified, giving a frequency of 0.128 for this allele (Table 3). Genotypic distributions in the 1990 year-class and previous year-classes differed in both the visible marker ($p < 0.0001$) and the *MDH-2** marker ($p < 0.01$). In contrast to the earlier year-classes, the 1990 year-class deviated significantly from the expected Hardy–Weinberg distribution in the pigmentation-controlling locus as well as in *MDH-2**. In 1992, homozygotes for the *MDH-2** marker allele were not detected in a

Fig. 2. Juvenile phenotypes from previous test matings corresponding to those found in the 1990 year-class in the River Øyreselv. Fine-spotted homozygotes, nos. 1 and 3 (from above); common, nos. 2 and 4; and heterozygotes, nos. 5, 6 and 7.

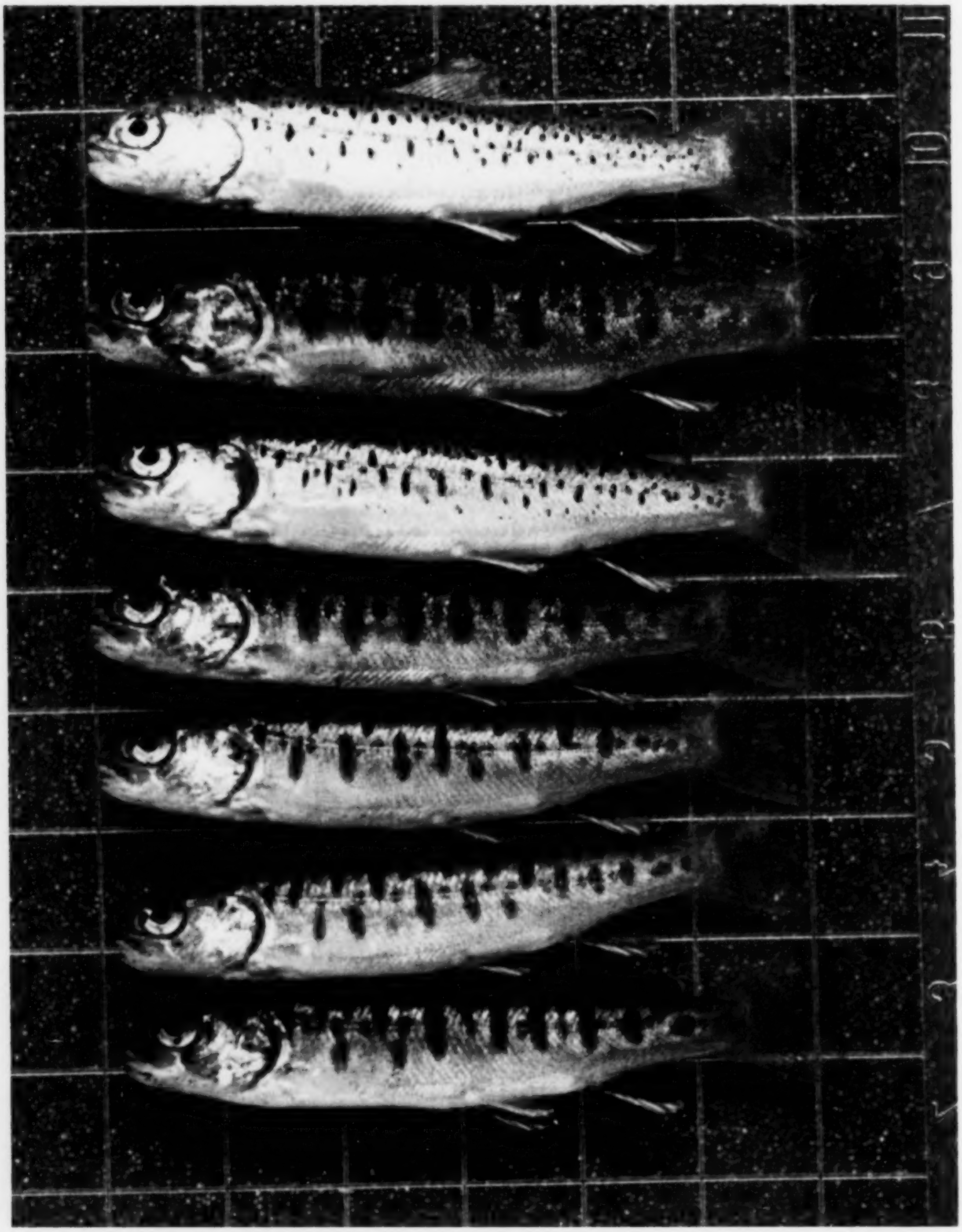


Table 2. Parameter values a and b in the model $q = aL^b$, the corresponding q values of 0+ parr and the average length of 0+ parr.

Date	a	b	r	q	Length (cm)*
Aug. 25, 1988	0.4031×10^{-3}	1.3763	0.9796	0.1129	6.0±0.7
Aug. 30, 1989	0.2875×10^{-5}	2.3586	0.9921	0.0335	5.2±0.6
Mar. 28, 1990	0.1789×10^{-3}	1.5058	0.9771	0.0788	5.8±0.8
Aug. 29, 1990	—	—	—	—	—
Feb. 27, 1991	0.1524×10^{-3}	1.5951	0.9727	0.0807	5.2±0.7
Aug. 28, 1991	0.9014×10^{-2}	0.7435	0.9391	0.1701	5.4±0.7
Mar. 5, 1992	0.1624×10^{-2}	1.0934	0.9391	0.1203	5.6±0.9
Sept. 2, 1992	0.1570×10^{-2}	1.1206	0.8225	0.1258	5.1±0.6
Feb. 18, 1993	0.6013×10^{-3}	1.2484	0.9714	0.0936	5.7±0.6

*Values are means ± SDs.

Table 3. Genotype and allele frequencies for the marker loci (*MDH-2** and *PIGM**) in section A, River Øyreselv, in the introduced strain and in different year-classes of the local wild stock.

		*MDH-2**				*PIGM**			
Sample and year-class	*N*	*100* *100*	*100* *152*	*152* *152*	*p* *152*	*WW*	*WF*	*FF*	*p* *f*
Hatchery 1987	50	1	18	31	0.800	0			
Wild 1985–1986	73	70	3	0	0.021	73	0	0	0.000
Wild 1988–1989	33	31	2	0	0.029	33	0	0	0.000
Wild 1990 (0+)	43	32	7	4	0.174	34	7	2	0.128
Wild 1990 (2+)	93	73	20	0	0.108	—	—	—	—

Note: The 1990 year-class was sampled in 1990 and 1992 as 0+ and 2+, respectively. *WW*, homozygous wild individuals; *WF*, heterozygotes; *FF*, homozygous non-indigenous individuals.

sample of 93 individuals, including 20 heterozygotes. Thus, the frequency of this allele had dropped to 0.108.

In section B, 0+ parr from the 1990 year-class carrying the genetic markers were detected in samples from 1990, 1991, and 1992 (Table 4). However, in this section the visible marker was only found in heterozygous form. In October 1990, 20 heterozygotes were recorded in this year-class, giving a frequency of the marker allele of 0.075, higher ($p < 0.0001$) than the frequency of this allele, (0) before the experiment. The frequency of the fine-spotted allele dropped significantly from 0.075 to 0.030 between October 1990 and February 1991. By June 1991 the frequency was 0.027.

The frequency of the *LDH-5** marker in the indigenous trout stock in section B varied from 0.027 to 0.034 in year-classes between 1986 and 1989, while in the 1990 year-class, this allele was found in 26 of 134 individuals, giving a frequency of 0.149, which is higher ($p < 0.0001$) than in previous year-classes. By June 1991, the frequency of the *LDH-5** marker had decreased to 0.106. Deviations observed from expected Hardy–Weinberg equilibrium were significant in both the pigmentation controlling locus and in *LDH-5**. In the 1992 sample, when the 1990 year-class had reached the 2+ stage, the frequency had dropped further to 0.092. Genotypic distributions differed between the 1990 year-class and the previous year-classes in both the visible marker ($p < 0.0001$) and in the *LDH-5** marker ($p < 0.0001$).

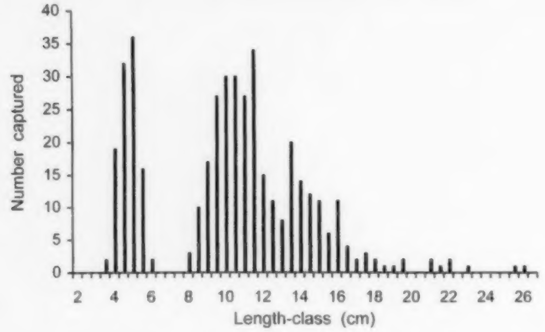
In section A, there was good agreement between the change in allelic frequencies estimated by the visible marker and the isozyme marker. The observed numerical change between the 1988–1989 year-classes and the 1990 year-class was 0.128 for the visible marker, and 0.144 for the isozyme marker (Table 3). In section B, the numerical change in allelic frequencies were 0.075 in the visible marker and 0.122 in the isozyme marker (Table 4). The change observed in the isozyme marker was, therefore, comparable with the change observed in section A, while the change observed in the visible marker was somewhat lower.

The genetic contribution of the introduced hatchery trout in section A by September 1990 was 19.2% based on the allozyme marker, *MDH-2**, and 12.8% based on the visible marker. In section B, the genetic contribution made by the introduced trout by September 1990, was 16.3% based on the allozyme marker *LDH-5**, and 7.5% based on the visible marker. By June 1991, the genetic contribution from introduced trout in section B had dropped to 10.9% according to the allozyme marker *LDH-5*100*, and to 2.7% according to the visible marker (Table 4).

Abundance of trout

The number of wild spawners in section A was estimated at approximately 100. In section B, the number of wild anadromous spawners with lengths between 25 and 65 cm, registered

Fig. 3. Length distribution of juvenile trout in section B, River Øyreselv.



during electrofishing, has been below 12 each autumn. Assuming a catchability of $q = 0.5$, the total number of wild spawners has been below 24, with the number of female spawners not exceeding 10–15. The individual lengths of the introduced spawners lay within the length range of wild spawners.

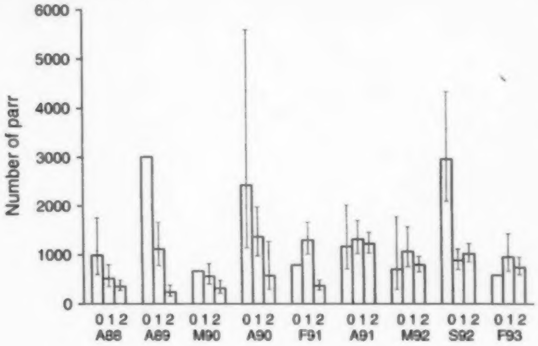
The anadromous trout in section B in River Øyre generally smolt at 3 years of age, at a length of 12–14 cm. Thus, few larger and older parr were present, and three modes can be identified in the length distributions (Fig. 3) corresponding to the 0+, 1+, and 2+ juveniles.

For single cohorts, the Petersen method and the CPUE method gave similar estimates of 0+ in section B (Fig. 4, Table 5). However, the abundance of 0+ parr shows considerable annual variation. The annual autumn numbers of 0+ varied from 800 to 3000, while the same cohorts in February–March had lower abundances and smaller annual variations in numbers, (400–700), indicating a high mortality rate from August–September to February–March in all years (Table 5).

From August 1990 to February 1991 the frequency of heterozygous 0+ decreased from 0.15 to 0.06, which gives a much lower survival rate of heterozygous 0+ compared with the indigenous 0+ cohort (Table 5). In the same period, the estimated survival rate of indigenous 0+, based on marked fish, was nearly identical to the survival rate based on the estimated number of fish (Table 5), indicating insignificant immigration of indigenous 0+ during the period August 1990 – February 1991. Thus, the lower survival of heterozygotes compared with indigenous 0+ is not explained by immigration of indigenous 0+. However, in August–September, the 1+ parr were more abundant than 0+ of the same cohort in the previous period (Fig. 4). From this it follows that immigration from the main river may occur from midwinter to the next autumn. Thus, due to the higher mortality rate of heterozygous 0+, and later on also due to immigration of indigenous trout, the frequency of introduced genetic material became increasingly lower.

The mean length of the 0+ parr in August 1988 was 6.0 ± 0.7 cm; ($n = 122$) compared with 5.2 ± 0.6 cm; ($n = 88$) in August 1989, and 4.5 ± 0.9 cm; ($n = 143$) in August 1990 (Table 2). In October 1990 the mean lengths of wild 0+ and of heterozygous 0+ resulting from crosses between wild and introduced trout were 4.7 ± 0.7 cm and 4.0 ± 0.6 cm, respectively, while in March 1991, there was no difference in size (5.1 ± 0.6 cm and 5.1 ± 0.3 cm, respectively) between the two groups.

Fig. 4. Number of 0+, 1+ & $ 2+ trout at the various sampling dates in section B, River Øyreselv. Vertical bars give SD for the Peterson estimates.



Discussion

Genetic impact

There was a considerable contribution of genetic material from the introduced hatchery trout in the F_1 generation, calculated at the 0+ stage, in both of the wild populations in the river. Thus, we demonstrated that not only did the non-indigenous hatchery trout engage in spawning activity, but also genetic material was actually incorporated in the gene pools of the wild anadromous and freshwater resident stocks. However, taking into account the relatively large number of spawners of non-indigenous origin compared with the number of wild spawners (about 4:1 in favour of non-indigenous spawners), the genetic contribution from non-indigenous population was less than expected (19.2 and 16.3% in sections A and B, respectively).

According to the population estimates and the genetic markers, we recorded a decline in the frequency and absolute number of individuals carrying non-indigenous alleles in the F_1 generation over about 2 years. The results suggest that the higher mortality rate observed in hybrid offspring may be caused by smaller size of individuals and lower capability to obtain and defend territories. The territorial behaviour of trout parr may result in a downstream drift of nonterritorial fish, which eventually die (Elliott 1986, 1987). Elliott (1989) found a critical survival time after fry emergence, and fry without territories decreased from a high number before the critical time to almost none afterwards. Although we have no data on the number of hatched fry and their mortality rate after hatching until August, the number of fry probably decreased considerably from spring to August. If the difference in survival rate from August to February was established already from the fry stage, the genetically marked fry may actually have been more abundant at time of hatching than offspring from wild fish. Thus, the declining frequency of the introduced genetic material in the F_1 generation may be due to a higher mortality rate of the heterozygotes compared to the native 0+. Immigration of parr to section B from the main river, above the branching

Table 4. Genotype and allele frequencies for the marker loci (*LDH-5** and *PIGM**) in section B, River Øyreselv, in the introduced strain and in different year-classes of the local wild stock. The 1990 year-class was sampled in 1990, 1991, and 1992 at the 0+, 1+, and 2+ stage, respectively.

	*LDH-5**					*PIGM**			
Sample and year-class	*N*	*90* *90*	*90* *100*	*100* *100*	*p* *100*	*WW*	*WF*	*FF*	*p* *f*
Hatchery 1987	50	3	21	26	0.730	0	0	50	1.000
Wild 1985–1988	102	95	7	0	0.034	73	0	0	0.000
Wild 1988–1989	55	52	3	0	0.027	55	0	0	0.000
Wild 1990 (0+)	134	101	26	7	0.149	114	20	0	0.075
Wild1990 (1+)	—	—	—	—	—	126	8	0	0.030
Wild 1990 (1+)	66	53	12	1	0.106	102	6	0	0.027
Wild1990 (2+)	60	49	11	0	0.092	—	—	—	—

Note: The 1990 year-class was sampled in 1990 and 1992 as 0+ and 2+ stage, respectively. *WW*, homozygous wild individuals; *WF*, heterozygotes; *FF*, homozygous non-indigenous individuals.

Table 5. Number and survival rates of indigenous (W) and hybrid (H) 0+ parr in section B.

Date	N W	N H	S_m W	S_n W	S_n H
Aug. 30, 1989	3150	0	—	0.208	—
Mar. 28, 1990	655	0			
Aug. 29, 1990	2060	364	0.371	0.351	0.130
Feb. 27, 1991	723	46			
Aug. 28, 1991	823	0	0.292	0.514	—
Mar. 5, 1992	423	0			
Sept. 2, 1992	2289	0	0.261	0.257	—
Feb. 18, 1993	566	0			

Note: Population numbers were obtained by the CPUE method (Borgstrøm and Skaala 1993). S_n, survival rate based on estimated number of 0+; S_m, survival rate based on recaptures of marked indigenous 0+ trout.

point, may further gradually reduce the frequency of heterozygotes from the 1+ stage. Therefore the genetic contribution in F_1 from the non-indigenous spawners may be regarded as a minimum estimate.

The genetically marked strain was originally derived from a population in a mountain lake at 1243 m above sea level. It has been documented that brown trout from lakes at high altitudes have smaller eggs than trout living at lower altitudes (Sømme 1941; Ø. Skaala, unpublished data). According to the length distribution of wild and heterozygous parr, there is a shift in the length distribution towards larger sizes between October 1990 and February 1991. The heterozygous 0+ parr were smaller in October than the wild 0+ parr, while their lengths were almost identical in February. Because the winter temperature in the river is low, it is unlikely that growth in length alone can explain the observed increase of about 1 cm in the mean length from October 1990 to February 1991 in 0+ parr. This may reflect size-specific mortality, i.e., higher mortality of the heterozygotes.

Genetic marking is a well established method that can be used to study gene flow (Gharrett and Seeb 1990). The genetic markers in the introduced hatchery trout stock enabled the gene flow from introduced to indigenous stocks to be recorded and quantified. In general, the visible marker was less effective than the isozyme markers in discriminating between alleles originating from introduced and wild stocks. The benefit of the visible marker, however, was that it permits nondestructive sampling and recording. An explanation for the difference between the visible and the biochemical genetic marker in section B, may be that heterozygotes for the visible marker are more easily misclassified than homozygotes are. Because of the lack of homozygotes in section B, this will be more important in section B than in section A. Therefore, the genetic contribution from introduced hatchery spawners in section B may be higher than indicated by the visible marker in this section. A few other experiments to estimate the reproductive success of reared fish have been conducted (Seeb et al. 1986; Skaala et al. 1990, and references therein; Crozier 1993; Moran et al. 1994; Crozier and Moffett 1995). Chilcote et al. (1986) used genetic marking to compare the reproductive success of transplanted, nonlocal hatchery steelhead trout (*Oncorhynchus mykiss*) with that of wild steelhead during the subyearling and smolt stages. They found that naturally spawning hatchery trout contributed substantially to the recruitment of wild juveniles. However, the reproductive success of their hatchery trout was considerably lower than that of the wild counterparts, as was also found in our study. Leider et al. (1990), who continued the work of Chilcote et al. (1986), found that the reproductive success of hatchery trout continued to decrease and was only 0.108–0.129 measured at the adult stage of the offspring.

Implications for management of aquaculture and wild stocks

The introduced spawners in our study were all first-generation hatchery fish without any experience with natural river environments, and limited physical conditioning compared with wild spawners. Nevertheless, the hatchery fish participated in spawning activities in both sections of the river and spawned among themselves as well as with individuals of the indigenous anadromous and freshwater resident trout stocks. The present experiment does not give any data on differences in spawning success or reproductive success between sexes. However, it is known from other studies on hatchery salmonids (Fleming and Gross 1993) that females have higher success than males, who have a very low success. In section B, there are indications that fry heterozygous for the marker loci are smaller than homozygous wild type, which is to be expected if mothers were of hatchery origin.

Although significant genetic changes were recorded in both of the indigenous trout populations, the number of genetically marked 0+ parr present in the river the year after spawning was much lower than expected from the number of genetically marked, introduced spawners, demonstrating a low spawning success of the introduced hatchery fish. Reduced performance and a low reproductive success of hatchery individuals in natural habitats may be caused by: (1) natural stock differences, (i.e., genetic differences between the stocks acquired through previous differentiation in natural environments), (2) genetic changes in the hatchery stock arising in the artificial environment, and (3) nongenetic factors, such as physiological stress or lack of stamina. In our study, the difference observed may be related to the pronounced genetic differences between the hatchery population and the wild stocks, differences in spawning behaviour and competitive ability of spawners, differences between reared and wild populations in the size of eggs and larvae, or behavioural differences of fry. As suitable spawning substrate in section B is limited, the released fish may have been forced to spawn in suboptimal habitats, with a higher risk of egg losses. It has been reported that escaped Atlantic salmon concentrate their spawning in the lower parts of rivers, which also may be a result of competition with wild spawners (Webb et al. 1991).

It is likely that the acclimatization and reproductive success of hatchery fish in natural environments depends on several circumstances, such as the life-stage at which the fish escape. For example, fish that escape at an early stage in life may suffer high mortality, but survivors have more time to acclimatize before spawning; therefore, survivors may reproduce more successfully than fish that escape as ripe spawners just before the spawning season.

Changes in productivity have been mentioned as one potential implication of introduction of non-indigenous genetic material (Crozier 1993). In our experiment there was a massive introduction of non-indigenous hatchery spawners in the spawning habitat just before the beginning of spawning in the wild population. Still, the presence of a high number of introduced spawners seems to have had little influence on the reproductive success of the wild anadromous brown trout, as the number of 0+ parr originating from wild parents was actually higher in August 1990 compared with the numbers in the two preceding years. Nevertheless, we have no information on the performance of the F_1 generation beyond the 2+ stage or on the F_2 generation.

Although the relative size of wild and hatchery eggs in our study deviates from the common situation, it serves to focus attention on a potentially important competitive point. Farmed salmonids are selectively bred for increased growth rate and size at maturity, which may imply that escaped spawners will be large compared with their wild counterpart. Thus, the size of eggs from farmed females will tend to be larger than eggs from wild females as there is a positive correlation between individual fish length and egg diameter (L'Abée-Lund and Hindar 1990). Given that this assumption is valid, fry hatched from farmed eggs will be larger than wild fry and thus have a competitive advantage at the early stage even though they may be less adapted at later stages. In conclusion then, hatchery fish may compete successfully at the spawning grounds and mate among themselves as well as with wild individuals. If a sufficiently high number of offspring in the F_1 generation compete successfully and survive, the outcome may be a change in the genetic characteristics of the wild populations. On the other hand, if their offspring compete less successfully, with a correspondingly high mortality in F_1, a whole year-class may be greatly reduced.

It is generally known that hybridization between populations can produce a wide range of effects from heterosis to outbreeding depression. There is still little empirical information on the magnitude of induced gene flow between wild and cultivated salmonid populations and on the biological consequences of it in terms of short-term or long-term changes in productivity (Waples 1991). From the present data we conclude that a single introduction of hatchery fish is not necessarily critical to the indigenous population, as the spawning success of hatchery fish may be low, and the survival rate of their offspring may be reduced due to a poor adaptation. However, when more frequent inputs of hatchery fish occur, as may often be the situation, the genetic characteristics of the wild stocks will be altered, even when the hatchery fish has a lower reproductive success. If the F_1 hybrids display increased fitness due to heterosis, the impact will be even greater.

Natural and induced fluctuations in population abundances involve complex mechanisms, such as changes in competition within and among cohorts, and in predation between age- and size-classes. It is highly probable that escapement and release of cultivated organisms in large numbers will affect wild populations ecologically and genetically, also through these mechanisms, in particular when the non-indigenous organisms breed successfully and produce unusually high densities of individuals in a year-class. If a numerous year-class is dominated by offspring from a non-indigenous stock, this year-class may prey heavily at least on the two consecutive year-classes. Thus, a numerous year-class with a high frequency of offspring from a non-indigenous source may lead to one or more weak year-classes and a wild population that is even more vulnerable to invading individuals.

Because the number of species and the biomass of cultivated organisms are rapidly increasing, it is likely that escapement and release of cultivated organisms is going to raise many more questions in the near future, in particular when taking into account development and potential commercial use of transgenic organisms. In the public as well as in the scientific community, there has been and will be public and scientific discussion about the usefulness and importance of preserving wild stocks. Many of the important questions within this discussion can only be answered through a combination of theoretical and empirical studies on stock discreteness and genetic adaptation in wild populations. For most species, such studies have only barely begun.

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Physiological effects of catch and release angling in Atlantic salmon (*Salmo salar*) at different stages of freshwater migration

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Abstract: The effects of exhaustive angling were investigated in one-sea-winter Atlantic salmon (*Salmo salar*) at two different stages of their freshwater migration. Physiological disturbances in kelts, returning to the ocean after a prolonged period of starvation, were compared with those of bright salmon, which had recently entered freshwater from the ocean. Condition factor was significantly higher in bright salmon than in kelts, and was associated with a greater time required to angle bright salmon to a state of exhaustion. White muscle concentrations of phosphocreatine and glycogen decreased in both kelts and bright salmon immediately after angling but recovered more quickly in kelts. White muscle lactate peaked after angling at 22.2 $\text{mmol}\cdot\text{L}^{-1}$ tissue water in kelts and at 40.6 $\text{mmol}\cdot\text{L}^{-1}$ tissue water in bright salmon. The intracellular acidosis that occurred in the white muscle following angling was also greater in bright salmon. Finally, mortality was not observed in any of the 24 angled kelts, but 3 of 25 bright salmon died within 12 h following angling. Together, these results demonstrate that the physiological disturbance from angling is smaller in kelts than in bright salmon.

Résumé : Nous avons étudié les effets de l'épuisement de saumons atlantiques (*Salmo salar*) unibermarins capturés à la ligne et fatigués par la pêcheur; ces poissons ont été pris à deux étapes de leur migration en eau douce. Nous avons comparé les déséquilibres physiologiques chez des charognards qui retournaient en mer après une longue période de jeune à ceux observés chez des saumons argentés qui avaient récemment pénétré en eau douce. Les saumons argentés étaient en bien meilleur état que les charognards et il fallait beaucoup plus de temps pour les épuiser après qu'ils aient mordu à l'hameçon. La concentration de la phosphocréatine et celle du glycogène dans le muscle blanc s'abaissaient immédiatement après la capture chez les deux groupes, mais se rétablissaient plus rapidement chez les charognards. La concentration du lactate dans le même tissu passait par un maximum de 22,2 $\text{mmol}\cdot\text{L}^{-1}$ d'eau tissulaire après la capture chez le charognard et de 40,6 $\text{mmol}\cdot\text{L}^{-1}$ d'eau tissulaire chez le saumon argenté. En outre, l'acidose intracellulaire mesurée dans le muscle blanc après la capture était plus marquée chez le argenté. Enfin, aucun des 24 charognards capturés n'est mort, mais 3 des 25 saumons argentés sont morts dans les 12 qui ont suivi leur capture. Au bilan, ces résultats montrent que les charognards sont moins perturbés par leur capture, sur le plan physiologique, que les saumons argentés.
[Traduit par la Rédaction]

Introduction

To reduce the negative impact of angling on wild Atlantic salmon (*Salmo salar*) stocks, mandatory catch-and-release was instituted about a decade ago for much of Canada's east coast salmon fishery. This policy requires that all angled multi-sea-winter (MSW) salmon (i.e., salmon >63 cm in fork length) must be released, while the voluntary release of smaller salmon (i.e., grilse; salmon <63 cm in fork length) is also promoted. At present, however, the biological implications of catch and release angling on the Atlantic salmon are not completely understood.

Several studies have shown that exhaustive exercise, such as that associated with angling, may lead to pronounced physiological disturbances that could contribute to significant postexercise, or "delayed" mortality of fish (Bouck and Ball 1966; Beggs et al. 1980; Graham et al. 1982; Wood et al. 1983). Wood et al. (1983) have suggested that the cause of delayed mortality may be related to the magnitude of the physiological disturbance within the white muscle of exhaustively exercised fish. In contrast, other investigators have demonstrated that significant mortality is not always a consequence of exhaustive exercise (Wydoski et al. 1976; Tufts et al. 1991; Booth et al. 1995).

During their freshwater spawning migration, which typically lasts from 6 months to a year, Atlantic salmon do not actively feed. Salmon that have spawned and are in the process of migrating back to sea are known as "kelts" or "black salmon," whereas those that have recently entered freshwater from their feeding grounds at sea are known as "bright

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salmon." At the present time, most Atlantic salmon angling seasons in eastern Canada are oriented towards the runs of bright salmon and the management policies regarding the angling of kelts are not consistent. It is currently illegal to intentionally angle for kelts in all Canadian provinces except Quebec and New Brunswick, where there are significant spring kelt fisheries in some rivers including the Miramichi River, New Brunswick, which is renowned for its large runs of Atlantic salmon. At times, even the legal angling of kelts in these rivers has been somewhat controversial because many people believe that angling and releasing these spent and starving salmon may lead to their mortality and, hence, negatively impact their potential reproductive contribution to future Atlantic salmon stocks. To date, however, the physiological consequences of catch and release angling in salmon at different stages of their freshwater migration (i.e., kelts vs. bright salmon) have not been investigated.

Starvation may have a profound impact on the energy reserves of fish (Ince and Thorpe 1976; Mommsen et al. 1980; Loughna and Goldspink 1984; Abe and Ohmama 1987; Jonsson et al. 1991; Scarabello et al. 1991; Martin et al. 1993). Stored lipids and protein are initially utilized to meet the energetic demands of migrating and starving salmonids (Mommsen et al. 1980; Cho and Kaushik 1985; Martin et al. 1993). It is noteworthy that decreased muscle protein levels in migrating salmon may exacerbate any acidosis resulting from exhaustive exercise, as protein is critically important in the non-bicarbonate buffering of muscle tissue (Castellini and Somero 1981). Starvation may also affect white muscle glycogen levels in fish (Ince and Thorpe, 1976; Scarabello et al. 1991), but the influence of starvation and (or) migration stage on glycogen stores in the Atlantic salmon are not currently known. White muscle glycogen levels largely determine the anaerobic capacity in fish and, hence, should also influence the magnitude of the physiological disturbance, and perhaps survival, following exhaustive exercise or angling.

During their residence in freshwater, Atlantic salmon are exposed to a wide range of ambient water temperatures. For example, water temperatures during the fall salmon and spring kelt angling seasons are generally less than 10°C. In contrast, water temperatures may exceed 20°C during the summer bright salmon angling season. Higher water temperatures appear to exacerbate the occurrence of delayed mortality in exercised Atlantic salmon (Wilkie et al. 1996; M.P. Wilkie, M.A. Brobbel, K. Davidson, L. Forsyth, and B.L. Tufts, unpublished data). Thus, water temperature may also be a controlling factor influencing the magnitude of the physiological disturbance and (or) recovery in angled kelts and bright salmon.

There is a clear need to assess the biological effects of catch and release angling in Atlantic salmon at different stages of their freshwater migration. Thus, we address the hypothesis that there will be significant differences in the physiological response to exhaustive angling between kelts and bright salmon; we also examine the short-term survival of these fish. It should be noted that the purpose of these experiments was to compare the physiological responses of these fish to angling under realistic field conditions. Thus, no attempt was made to control for the exogenous (e.g., temperature) or endogenous (e.g., nutritional and osmoregulatory status) factors that differ between these migratory stages of Atlantic salmon.

Materials and methods

Animals

Kelts ($N = 30$) of grilse size (fork length less than 63 cm) were collected from the Miramichi River near Miramichi, N.B. (May 4–11, 1994) during their return migration to the Atlantic Ocean. These kelts were caught using heavy spinning gear and landed as quickly as possible (typically in under 2 min) to minimize burst activity due to angling. The fish were kept in live boxes sitting in running river water (water temperature 5 ± 2°C) for no more than 24 h before they were transported to the Miramichi Fish Culture Station, South Esk, N.B.

Bright Atlantic salmon ($N = 32$), grilse size, were obtained from Department of Fisheries and Oceans' box traps (18 × 3 × 5 m, used for assessment purposes) about 1 km below the head of tide in the freshwater portion of the Main Southwest Miramichi River near Millerton, N.B. (July 5–12 1994) during the initial stages of their upriver migration (water temperature 19 ± 2°C). These bright salmon were also transported to the Miramichi Fish Culture Station for the angling experiments.

Experimental protocol

Kelts were kept in large, dimly lit fish tanks for at least 24 hours before they were transferred individually a short distance (approx. 100 m) to compartmentalized wooden fish boxes at Stuart Brook (a small tributary of the Northwest Miramichi which is the water source for the Miramichi Fish Culture Station), where they were held for at least another 12 h prior to the experiments. The streamside wooden boxes were gravity fed with a continuous supply of fresh Stuart Brook water (4 ± 1°C).

The bright salmon were transferred directly from the oxygenated transport tanks to the wooden fish boxes at Stuart Brook on the grounds of the Miramichi Fish Culture Station where they were held for a minimum of 24 h, but for no longer than 36 h, in order to control for the duration of their stay in freshwater. This was done to best mimic the angling of bright salmon shortly after they enter freshwater from the sea. The mean temperature of Stuart Brook during the bright salmon period of experimentation was 16 ± 1°C.

As in Booth et al. (1995), control fish for both the kelt ($N = 6$) and bright salmon ($N = 7$) experiments were individually anaesthetized using tricaine methanesulfonate (MS-222) at a final concentration of $0.25\ g \cdot L^{-1}$ buffered with $NaHCO_3$ ($0.5\ g \cdot L^{-1}$). Water flow through the boxes was stopped at this time to allow the anaesthetic to take effect at a faster rate. Once anaesthetized, individual fish were removed from their compartments and 5–8 mL venous blood samples were taken by caudal puncture. The blood was then centrifuged for 4 min to separate the plasma from the red blood cell pellet, after which 500 μL of plasma was added to an equal volume of 8% perchloric acid in a 1.5 mL Eppendorf tube. All plasma samples were immediately frozen in liquid nitrogen for later analyses. After blood sampling, the fish were quickly weighed before a sample of white muscle was removed (approximately 20 g) from the epaxial musculature anterior to the dorsal fin. The muscle was then freeze-clamped in liquid nitrogen, wrapped in tin foil and stored in liquid nitrogen for later analyses. According to Wang et al. (1994), this method is most appropriate to obtain tissue samples from fish for metabolite analysis. The time elapsed between fish removal from the water and tissue sampling was about 30 s. The fish were then killed immediately by decapitation while they were still anaesthetized.

In contrast to the controls, the remaining grilse in each experiment were individually hooked in the lower jaw with a barbed fly hook, removed from the holding box, and transferred to Stuart Brook (width approx. 5 m) where they were played to exhaustion using fly fishing gear. After each individual fish was angled to a state of exhaustion (i.e., ceased fighting and did not respond to increased line tension), it was transferred by hand less than 1 m to its original compartment in the holding box where it was unhooked and allowed to recover in flowing brook water until later anaesthetization and sampling as described for control fish. Groups of recovering fish were also anaesthetized, sampled, and then killed as described above for the control fish at 2, 4, or 12 h following exercise ($N = 6$ for each of these sampling times and for each type of salmon).

Another group of kelts ($N = 6$) and bright salmon ($N = 7$) were sampled immediately following exercise. Once angled, these fish were individually set in a 40-L Plexiglas fish box containing buffered MS-222 solution at a final concentration of 0.25 $g \cdot L^{-1}$ and they were then sampled according to the protocol described above.

Analyses

Condition factor

The condition factor (K) of each salmon was determined according to the formula provided by Hunn and Greer (1991):

$$K = \frac{(W \times 10^5)}{L^3}$$

where W is wet mass of the fish (grams), and L is total length of the fish (millimetres).

Muscle water content

Muscle tissue (approx. 200–500 mg) was added to a dried and tared 1.5-mL Eppendorf tube and its wet mass was determined. The samples were placed in an oven at 80°C and dried until a constant mass was obtained. Water content was then calculated using the following equation:

$$H_2O\ (\%) = 100 - \left(\frac{100 \times \text{dry mass}}{\text{wet mass}}\right)$$

Since the muscle water contents were significantly different ($P < 0.05$, unpaired t-test) between kelts and bright salmon, the white muscle metabolite concentrations were corrected for water content and presented in terms of millimoles per litre of tissue water (tw) by dividing the wet tissue metabolite concentration (millimoles per kilogram wet mass) by the fraction of tissue water for each muscle sample.

Muscle extraction and metabolite levels

Samples of salmon white muscle were prepared for enzymatic analysis according to the protocol described by Booth et al. (1995). Phosphocreatine (PCr), free creatine, adenosine triphosphate (ATP), and lactate concentrations of the samples were then determined on neutralized extracts using the methods outlined by Bergmeyer (1983). Samples were analyzed for glycogen according to the method of Hassid and Abraham (1957). The total creatine pool was estimated by adding white muscle PCr and free creatine concentrations.

Muscle acid–base status

White muscle acid–base status was determined according to the method of Pörtner et al. (1990) and as outlined by Kieffer et al. (1994). To determine total CO_2 (C_{CO_2}), 100 μL of supernatant was added to a Corning Model 965 CO_2 analyzer (CIBA Corning Canada Inc.) with a Hamilton gas-tight syringe. The pH of approximately 100 μL of supernatant was measured using a PHM 73 pH meter and micro-pH unit (Radiometer, Copenhagen, Denmark) thermostatted to the appropriate temperature (4°C for kelts; 16°C for bright salmon). Means of C_{CO_2} and pH were calculated from duplicate measurements. Tissue water C_{CO_2} was then calculated using the equation provided by Pörtner et al. (1990) and values for pK' based on temperature and pH were determined for each fish according to the equation of Boutilier et al. (1984). The postexercise metabolic proton load (ΔH^+_m) was calculated using the equation of Milligan and Wood (1986):

$$\Delta H^+_m = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta(pH_1 - pH_2)$$

where the subscripts 1 and 2 represent $[HCO_3^-]$ and pH at rest and following a period of recovery, respectively, and β is the non-bicarbonate buffer value (see below).

Muscle non-bicarbonate buffer value

Non-bicarbonate buffer values were determined using the method of Pörtner (1990). Approximately 2 g of muscle tissue taken from kelt and bright salmon control fish was ground under liquid nitrogen and placed in 8 mL of ice-cold metabolic inhibitor solution (150 $mmol \cdot L^{-1}$ potassium fluoride; 6 $mmol \cdot L^{-1}$ nitrilotriacetic acid). The homogenate was vortexed and placed into precooled tonometer vessels. Samples were then equilibrated at their respective acclimation temperatures for 45 min at P_{CO_2} levels of 1, 2, 5, and 9% in air using a Wösthoff gas-mixing pump (Bochum, Germany). After each equilibration period, 1-mL samples were removed and centrifuged for 4 min in Eppendorf tubes. Samples were then analyzed for pH and C_{CO_2} as described above. Bicarbonate concentration was calculated from C_{CO_2} values using a solubility coefficient of CO_2 obtained from the equation provided by Boutilier et al. (1984). The non-bicarbonate buffering capacity (β) for each fish was then determined by plotting $[HCO_3^-]$ against pH and measuring the slope of the straight line.

Plasma metabolites and ions

Plasma samples were thawed and analyzed for lactate as described by Lowry and Passonneau (1972) using Sigma reagents and the appropriate standards. Plasma chloride concentrations were determined with a CMT10 chloride titrator (Radiometer, Copenhagen, Denmark) and sodium and potassium concentrations were measured using a Corning model 410C clinical flame photometer (CIBA Corning Canada Inc.), using the appropriate standards.

Statistics

Values have been reported as means ± 1 SE. A one-factor analysis of variance (ANOVA) was used to assess significant differences between sample times in both kelt and bright salmon experiments. A Scheffe's F-test ($P < 0.05$) was then used to determine whether there were significant differences between control values and the postexercise values at any given time. Kelt and bright salmon mean values were compared at any given sample time using unpaired t-tests. In addition, unpaired t-tests were used to investigate significant differences between kelts and bright salmon with respect to their mean non-bicarbonate buffer values, condition factors, and angling times.

Results

As expected, the stage of freshwater migration influenced the condition factor in Atlantic salmon, as bright salmon had significantly higher condition factors ($K = 0.926 \pm 0.01$) than kelts ($K = 0.534 \pm 0.01$). In addition, the time required to angle the fish to exhaustion was greater in bright salmon (320 ± 26 s) than in kelts (187 ± 11 s).

At all sample times, the water content of kelt white muscle was markedly higher than that of bright salmon (Table 1). However, angling did not cause changes in muscle water content in either kelts or bright salmon (Table 1). White muscle total creatine concentrations remained stable around 75 $mmol \cdot L^{-1}$ tw in both kelts and bright salmon, demonstrating that the total creatine pool was not influenced by the stage

Table 1. White muscle water content in kelts and bright salmon before (at rest) and after angling.

Sampling time	Kelt water content (%)	Bright salmon water content (%)
Rest	81.1±0.2*	60.6±2.5
0 h	80.6±0.3*	61.9±3.2
2 h	82.2±0.4*	65.3±2.4
4 h	80.6±0.8*	62.4±2.2
12 h	82.1±0.2*	68.5±3.7

*Significant differences ($P < 0.05$) between kelts and bright salmon at each sampling time. There were no significant differences between different sample times within either group.

of freshwater migration. Immediately following angling, the concentration of white muscle PCr was decreased by 57% in kelts and 25% in bright salmon, but PCr had recovered to control levels by 2 and 4 h, respectively (Fig. 1A). Muscle ATP levels in bright salmon were significantly higher than those in kelts at each sample time, but ATP concentrations did not drop significantly below control values in either group of salmon after angling (Fig. 1B). At rest, the glycogen content of kelt white muscle was 44% lower than that of bright salmon (Fig. 1C). Glycogen levels remained significantly lower than control values until the 2-h recovery sample in kelts, and until the 12-hour recovery sample in bright salmon (Fig. 1C).

White muscle lactate levels were significantly elevated following exercise in both groups of salmon, peaking at 22.2 ± 1.0 $\text{mmol}\cdot\text{L}^{-1}$ tw and 40.6 ± 3.6 $\text{mmol}\cdot\text{L}^{-1}$ tw in kelts and bright salmon, respectively (Fig. 2A). Muscle lactate levels returned to values that were not significantly different from control values by 2 h in kelts but not until 4 h in bright salmon (Fig. 2A). Plasma lactate concentration increased significantly from resting levels in both groups (Fig. 2B). In bright salmon, the plasma lactate concentration peaked at 2 h post-exercise and did not decrease to control levels until the 12-h sample time (Fig. 2B). In contrast, plasma lactate levels in kelts were only significantly higher than control values immediately after angling and following 2 h of recovery (Fig. 2B).

The metabolic proton load (ΔH^+_m) peaked following angling at 35.0 ± 4.4 and 47.6 ± 5.1 $\text{mmol}\cdot\text{L}^{-1}$ tw in kelts and bright salmon, respectively (Fig. 3A). Muscle bicarbonate concentration decreased by 36% in bright salmon after angling but was not significantly different from control values at any sample time in kelts (Fig. 3B). The β values of the white muscle were 93.1 ± 3.3 and 98.1 ± 3.5 $\text{mmol}\cdot\text{L}^{-1}\cdot\text{pH unit}^{-1}$ for kelts and bright salmon, respectively, and were not significantly different. Angling caused white muscle intracellular pH (pH_i) to fall from about 7.4 to 7.1 in kelts and from 7.2 to 6.7 in bright salmon, and it recovered to control values by 2 and 4 h, respectively (Fig. 3C). Kelt muscle pH_i was significantly higher than that of bright salmon muscle at rest and at all postangling sample times (Fig. 3C).

Plasma Na^+ and K^+ concentrations increased markedly in kelts immediately following angling, but only K^+ increased in bright salmon plasma at this sampling time (Table 2).

None of the angled kelts died, but 3 of 25 bright salmon died within 12 hours following angling.

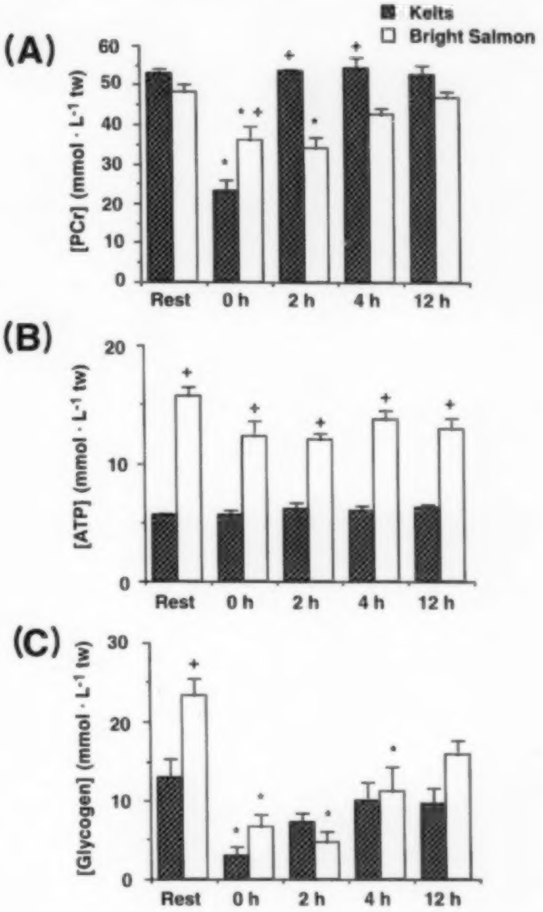


Fig. 1. PCr (A), ATP (B), and glycogen (C) concentrations ($\text{mmol}\cdot\text{L}^{-1}$ tw) in the white muscle of kelts and bright salmon at rest and following 0, 2, 4, and 12 h of recovery after angling. Asterisks denote significant differences ($P < 0.05$) from resting values and plus signs, significant differences ($P < 0.05$) between kelts and bright salmon at each sample time. ($N = 6$ for all sampling times, except $N = 7$ for bright salmon at rest and at 0 h, and $N = 3$ for bright salmon after 12 h of recovery).

Discussion

The results of this study indicate that the stage of freshwater migration had a large influence on the magnitude of the physiological disturbance in Atlantic salmon following exhaustive angling. The capacity for exhaustive exercise, the magnitude of the postangling disturbance, and the likelihood of mortality following angling were all greater in bright salmon than in kelts. These results can probably be best explained by the combined influence of several specific factors that differ between these migratory stages.

It is likely that the different degrees of starvation between bright salmon, which have only recently stopped feeding upon entering freshwater, and kelts, which have not fed for

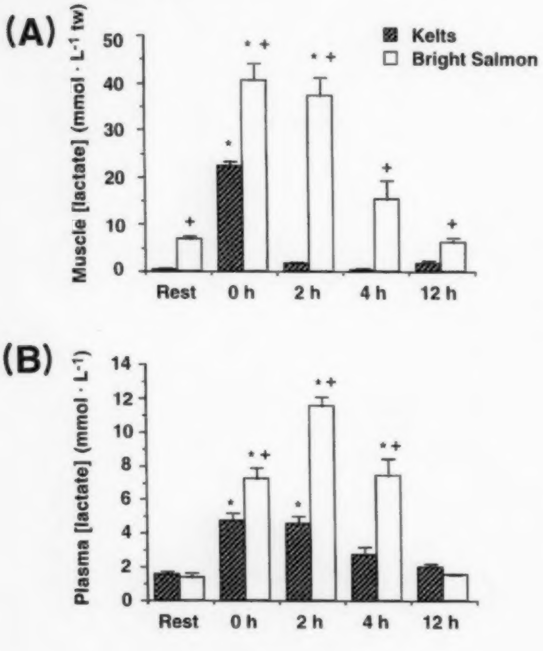
Table 2. Changes in plasma ion concentrations in kelts and bright salmon at rest and at different recovery times following angling.

Group	Sampling time	[Na^+] (mequiv.·L^{-1})	[K^+] (mequiv.·L^{-1})	[Cl^-] (mequiv.·L^{-1})
Kelts	Rest	126.5±3.2	1.8±0.2[b]	114.6±4.2
	0 h	152.0±5.3[a,b]	3.7±0.1[a,b]	135.1±4.3[b]
	2 h	121.1±4.8	2.4±0.3[b]	113.3±5.5[b]
	4 h	117.6±4.3	2.4±0.3[b]	108.4±5.5
	12 h	130.8±1.1[b]	2.3±0.1[b]	116.2±1.0[b]
Bright salmon	Rest	123.0±6.2	3.2±0.3	105.6±6.0
	0 h	119.3±3.1	4.9±0.2[a]	104.5±2.4
	2 h	119.0±4.4	4.0±0.4	91.8±4.0
	4 h	114.7±6.1	4.6±0.4	90.6±5.9
	12 h	96.0±6.0	3.4±0.1	82.3±4.7

[a] Significant differences ($P < 0.05$) from resting values.

[b] Significant differences ($P < 0.05$) between kelts and bright salmon.

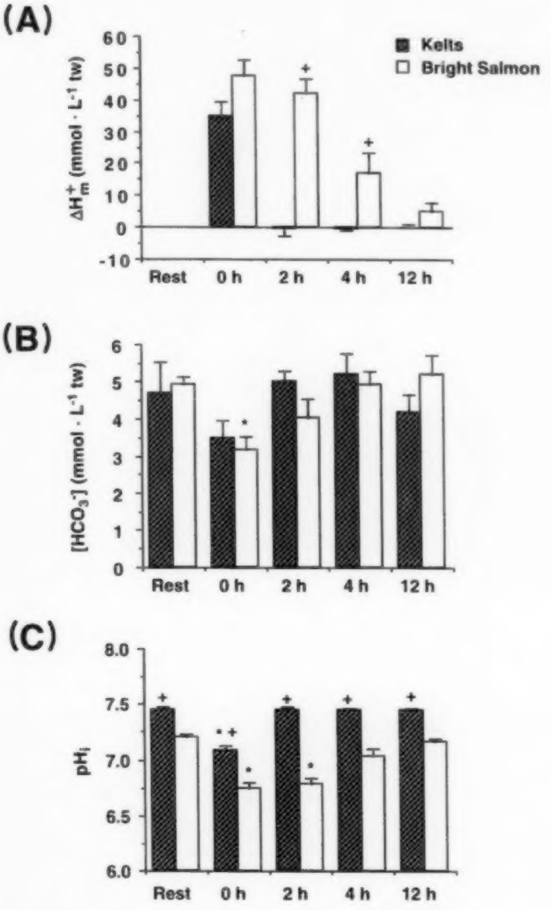
Fig. 2. White muscle lactate (A) and plasma lactate (B) concentrations of kelts and bright salmon at rest and following 0, 2, 4, and 12 h of recovery after angling. Asterisks denote significant differences ($P < 0.05$) from resting values and plus signs, significant differences ($P < 0.05$) between kelts and bright salmon at each sample time. Other details are as in Fig. 1.



almost a year, largely account for the different physiological responses to angling observed in these two groups of salmon. The significantly different condition factors and muscle water contents obtained for the bright salmon support the view that starvation is indeed a critical factor affecting the physiological status of Atlantic salmon at different migratory stages. The bright salmon in our study had condition factors comparable with those previously reported for recently feeding July-captured Atlantic salmon (Martin et al. 1993) and laboratory-reared juvenile Atlantic salmon (Hunn and Greer 1991), whereas much lower condition factors were observed in the thoroughly starved kelts. The higher water content of kelt white muscle (about 20%) at each sample time is also best explained by the fact that these fish have been deprived of food for a much longer period of time than the bright salmon. Several previous studies have demonstrated that starvation increases the water content of fish muscle (Love 1970; Moon and Johnston 1981; Morata et al. 1982; Black and Love 1986).

The concentrations of white muscle glycogen, lactate, and ATP were also markedly influenced by migratory stage and could probably be best explained by the different degrees of starvation in bright salmon and kelts. The fact that glycogen concentrations in kelt white muscle at rest were 44% lower than those in bright salmon suggests that the prolonged period of starvation in kelts had a profound impact on their white muscle carbohydrate stores. Similar starvation effects have also been observed in other fish species. For example, muscle glycogen levels in northern pike (*Esox lucius*) decreased by about 60% following 3 months of starvation (Ince and Thorpe 1976). In addition, Scarabello et al. (1991) found that whole body glycogen stores of rainbow (*Oncorhynchus mykiss*) trout decreased by 52% following only 5 days of starvation. According to Wardle (1978), there should be a 1:2 ratio between glycogen depletion and lactate production following exhaustive exercise. Thus, the lower lactate levels in kelt muscle after angling would be expected because their white muscle glycogen stores at rest were lower than those in bright salmon. The present results, when combined with those of Booth et al. (1995), also strongly indicate that long-term starvation causes a reduction in white muscle ATP values in Atlantic salmon. To our knowledge, such effects of long-term starvation on muscle ATP levels in fish have not previously been documented. Taken together, our white muscle metabolite results suggest that the degree of starvation probably has a profound influence on the anaerobic capacity of Atlantic salmon at different migratory stages. Not surprisingly, the time to angle kelts to exhaustion was much shorter than that

Fig. 3. Metabolic proton load, ΔH_m^+ (A), bicarbonate concentration (B), and intracellular pH (C) in the white muscle of kelts and bright salmon at rest and following 0, 2, 4, and 12 h of recovery after angling. Asterisks denote differences ($P < 0.05$) from resting values, and plus signs denote significant differences ($P < 0.05$) between kelts and bright salmon at each sample time. Other details are as in Fig. 1.



for the bright salmon. Furthermore, the time required for most physiological variables to recover to resting levels was also generally much less in kelts, probably since the magnitude of the physiological disturbance after angling was initially lower than that in bright salmon.

The differences in plasma ion concentrations between bright salmon and kelts during recovery from angling suggest that there were also important differences in the osmoregulatory abilities of these two groups of salmon. The drastic changes in ambient salt levels that anadromous species are exposed to during the transition from the ocean to freshwater is known to be an important osmoregulatory stress (McKeown 1984). Since the bright salmon used in this study were collected from a trap that was in freshwater, but still below the head of tide, it is possible that these fish had not fully adjusted to freshwater prior to angling. It should be noted that it was our intended purpose to examine the effects of angling in bright salmon that had only recently entered freshwater, and that may be in this transitional osmoregulatory state, since this probably reflects the status of many bright salmon that are angled under natural conditions in the wild. In contrast, the kelts used in this study had already spent many months in freshwater prior to our experiments. Freshwater salmonids normally experience a net loss of sodium and chloride across the gills immediately following exhaustive exercise, but increased branchial uptake of these ions contributes to the restoration of ionic balance later in the recovery period (Wood 1988, 1991). Moreover, plasma ion concentrations following exercise often show little or no evidence of these branchial events because postexercise shifts of water and ions between the extracellular and intracellular fluid will also influence the levels of these ions measured in the plasma (Wood 1991). The observed changes in plasma sodium and chloride levels in kelts are typical of the changes found in other freshwater salmonids following exhaustive exercise. In contrast, the changes in these ions in bright salmon suggest that salmon that have only recently entered freshwater may be unable to fully restore ionic balance following a bout of activity as severe as angling. In bright salmon, plasma sodium and chloride levels continue to fall after angling and become significantly lower than those in kelts. From our data, it is impossible to determine whether this is due to increased ion losses (i.e., increased branchial permeability) across the gills of the bright salmon, reduced capacity for branchial ion uptake, or a combination of both. Since branchial ion transport mechanisms also play an important role in acid–base regulation (McDonald et al. 1989; Wood 1991), however, it is possible that these branchial limitations may have also contributed to the slower recovery of acid–base status in bright salmon after angling.

Several aspects of the physiological responses of salmon angled at these different migratory stages may have been influenced by temperature. For example, higher temperatures would be expected to increase the net losses of sodium and chloride across the gills after exhaustive exercise (Kieffer and Tufts 1996). In bright salmon, this effect of temperature probably contributed to their inability to restore ionic balance following angling. Although environmental temperature does not appear to influence the anaerobic capacity of salmonids (Kieffer and Tufts 1996: M.P. Wilkie, M.A. Brobbel, K. Davidson, L. Forsyth, and B.L. Tufts, unpublished data), the rate of recovery after exercise is generally enhanced in Atlantic salmon in warmer water (M.P. Wilkie, M.A. Brobbel, K. Davidson, L. Forsyth, and B.L. Tufts, unpublished data). In the present study, the fact that the warmer bright salmon seemed to recover more slowly than the colder kelts suggests, however, that other factors probably had a more important influence on the rate of physiological recovery in salmon angled at these different migratory stages.

The mortalities observed in three of the bright salmon after angling undoubtedly result from the greater postangling physiological disturbance in these fish. Wood et al. (1983) suggested that mortality in severely exercised fish is probably related to the magnitude of the intracellular acidosis within the white muscle. The present results are consistent with this explanation, since the magnitude of the white muscle acidosis (ΔH_m^+, pH_i) in bright salmon was greater than that in kelts where no postangling mortalties were observed. It is likely that the disturbances to plasma electrolyte balance also contributed to the observed postangling mortality in bright salmon. As mentioned earlier, the higher environmental temperature experienced by the bright salmon would also be expected to exacerbate ion losses across the gills following exercise (Kieffer and Tufts 1996). In fish exposed to acid water, decreases in plasma ions that are similar in magnitude to those observed in bright salmon are thought to contribute to the cause of death (i.e., ionoregulatory failure; Wood 1989). Other studies have also shown that mortalities are increased when Atlantic salmon are exhaustively exercised or angled in warmer water but not until temperatures reach about 22°C (Wilkie et al. 1996; M.P. Wilkie, M.A. Brobbel, K. Davidson, L. Forsyth, and B.L. Tufts, unpublished data).

In summary, it appears that several factors, such as the degree of starvation, osmoregulatory status, and environmental temperature probably influence the physiological responses to angling in Atlantic salmon at different migratory stages. The combined effects of these factors contribute to smaller physiological disturbances from angling in kelts than in bright salmon. Since there were also no mortalities among the kelts angled in these experiments, our study provides support for catch and release spring kelt fisheries in Atlantic Canada. Our results also confirm that "catch-and-release" can potentially be an extremely useful management strategy for the enhancement of wild Atlantic salmon stocks.

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Seasonal variation of lipid composition, weight, and length in juvenile *Diporeia* spp. (Amphipoda) from lakes Michigan and Ontario[1,2]

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Abstract: Benthic amphipods, *Diporeia* spp., were collected during 1988 and 1989 at four sites to compare size and lipid levels of animals living in Lakes Michigan and Ontario: sites were at depths of 45 and 100 m in Lake Michigan and 35 and 125 m in Lake Ontario. In Lake Michigan, the mean length, mass, and lipid levels of individual *Diporeia* from 45 m were significantly different from those collected from 100 m in 1988; in 1989, only mass and lipid levels were significantly different between depths. In contrast, regardless of the year, the Lake Ontario *Diporeia* from 35 m were similar in mass and length to those from 125 m, but lipid levels were significantly different between depths. Combined means for total lipid for each lake over similar months revealed higher levels in Lake Ontario than in Lake Michigan *Diporeia*. Triacylglycerols were the dominant lipid class in all the amphipods, with mean levels reaching a maximum of 84% of the total lipid in individuals from Lake Ontario at 35 m. Trophic state, food-web, and (or) genetic differences are probably responsible for the occurrence of higher lipid levels in Lake Ontario *Diporeia* than in Lake Michigan *Diporeia*.

Résumé : Des amphipodes benthiques, Diporeia spp., ont été prélevés en 1988 et 1989 dans quatre sites pour comparer la taille et la teneur en lipides des animaux vivant dans les lacs Michigan et Ontario. Les sites de prélèvement se situaient à 45 et 100 m dans le cas du lac Michigan et à 35 et 125 m dans le cas du lac Ontario. Dans le lac Michigan, les longueurs, masses et concentrations en lipide moyennes des *Diporeia* individuels vivant à la profondeur de 45 m différaient de manière statistiquement significative de celles des organismes prélevés à 100 m en 1988; en 1989, seules la masse et la concentration en lipide différaient de manière significative d'une profondeur à l'autre. À l'opposé, peu importe l'année, les *Diporeia* du lac Ontario prélevés à une profondeur de 35 m étaient semblables par leur taille et leur masse à ceux qui ont été prélevés à 125 m, mais leur concentration en lipide était différente de manière significative. Les moyennes combinées des lipides totaux pour chacun des lacs au cours des mêmes mois ont révélé des concentrations plus élevées dans les *Diporeia* du lac Ontario que dans ceux du lac Michigan. Les triacylglycérols constituaient la classe de lipide dominante chez tous les amphipodes, les concentrations moyennes atteignant un maximum de 84 % des lipides totaux chez les animaux du lac Ontario vivant à 35 m. L'état trophique, le réseau alimentaire et (ou) des différences génétiques expliquent probablement les concentrations en lipide plus élevées observées chez les *Diporeia* du lac Ontario comparativement à ceux du lac Michigan.
[Traduit par la Rédaction]

Introduction

The amphipods *Diporeia* spp. are the most abundant macrobenthic organisms in the slope and profundal zones of the Great Lakes (Johannsson et al. 1985; Nalepa 1987; Evans et al. 1990) and are a direct link between spring primary production and fish. Previous studies have shown that *Diporeia* feeds directly on the spring diatom bloom after it settles to the lake bottom. For instance, fragments of the diatoms *Cyclotella* spp. and *Melosira* spp. are the most common biological remains found in *Diporeia* guts (Evans et al. 1990) and the frequency of full guts is highest in the spring, implying active feeding on fresh food inputs at this time (Quigley 1988; Dermott and Corning 1988). *Diporeia* lipid levels increase after

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Table 1. Summary of sample collections from Lakes Michigan at 45 and 100 m and Lake Ontario at 35 and 125 m during 1988 and 1989.

	Lake Michigan		Lake Ontario	
Date	45 m	100 m	35 m	125 m
1988				
19 Apr.			d,f,b	d,b
21 Apr.	d,b,f,l	d,b,l		
10 May			d,f,b	
12 May	d,b,f,l	d,b,l		
01 June			d,b,f,l	l
08 June	d,b,f,l	d,b,l		
21 June			d,b,f,l	l
29 June	d,b,f,l	d,b,l		
12 July	d,b,f,l	d,b,l		
20 July			d,b,f,l	d,b,l
17 Aug.			l	l
19 Aug.	l			
29 Aug.	d,b,f,l	d,b,l		
12 Sept.			d,b,f,l	l
12 Oct.			d,b,f,l	d,b,l
14 Nov.	d,b,l	d,b,l		
1989				
18 Apr.	d,b,l	d,b,l		
25 Apr.			d,b,f,l	d,b,l
15 May	d,b,l	d,b,l		
18 May			l	l
14 June			l	l
15 June	d,b,l	d,b,l		
02 Aug.			d,b,l	d,b,l
23 Aug.	d,b,l	d,b,l		
11 Oct.			d,b,l	d,b,l

*d, density; b, biomass; f, frequency distribution; l, lipid.

the diatom bloom in Lake Michigan, suggesting their use of this high-quality food source (Gardner et al. 1985*a*, 1990). In turn, *Diporeia* is fed upon by numerous species of fish (Wells 1980; McDonald et al. 1990).

A direct link between primary production and benthic biomass often occurs in temperate large lakes (Gardner et al. 1990; Johnson and Wiederholm 1992). Although primary production and sedimentation rates are higher in Lake Ontario than in Lake Michigan, benthic biomass is greater in Lake Michigan (Nalepa 1991). This inconsistency between Lakes Michigan and Ontario has caused some speculation about the nature of the pelagic–benthic coupling systems in these two lakes. Differences in fish predation, levels of persistent contaminants, and SiO_2 concentrations have all been suggested to explain differences in benthic biomass between the two lakes (Nalepa 1991; Sly and Christie 1992). Because lipid levels and lipid classes are an excellent indicator of the nourishment available to *Diporeia*, we conducted a seasonal study of lipids in *Diporeia* in Lakes Michigan and Ontario to examine pelagic–benthic coupling in the two lakes. *Diporeia* were examined at a sublittoral and profundal site in each lake.

Diporeia spp. were formerly known as one species, *Pontoporeia hoyi*, in the Great Lakes ecosystem; however, the genus *Diporeia* contains at least two and perhaps as many as eight species (Bousfield 1989). Because this study was undertaken when these amphipods were all classified as *Pontoporeia hoyi* we will simply group them under the genus *Diporeia* with no specific designation.

Methods

Diporeia were collected spring through fall in 1988 and 1989 from a 45-m site (43°1′N, 86°20′W) and a 100-m site (43°1′N, 86°37′W) in southern Lake Michigan offshore from Grand Haven, Mich., and a 35-m site (43°59′N, 76°39′W) in Lake Ontario's eastern basin off Main Duck Island and a 125-m site (43°43′N, 78°02′W) in the middle of Lake Ontario. On dates specified in Table 1, benthic organisms were collected with a Ponar grab, washed through a 500-μm screen for the Lake Michigan samples or a 250-μm screen for the Lake Ontario samples, and preserved in 10% formalin for later enumeration and length measurements to determine *Diporeia* areal density and biomass. As many as 75% of newly hatched young-of-the-year (YOY) may pass through a 500-μm screen (Johnson 1988); hence, we corrected for the different screen sizes used in each lake by subtracting the proportion of 1–2 mm amphipods determined for each month. Lengths were converted to biomass using an empirical relationship that was derived from a subset of unpreserved *Diporeia*. The Lake Michigan biomass estimates are conservative because they were based on the non-normalized 500-μm screen densities. *Diporeia* size–frequency distributions (1-mm size categories excluding the

Fig. 1. Mean lengths of *Diporeia* from Lake Michigan and Lake Ontario that were examined for total lipid and lipid classes. Error bars are SEs.

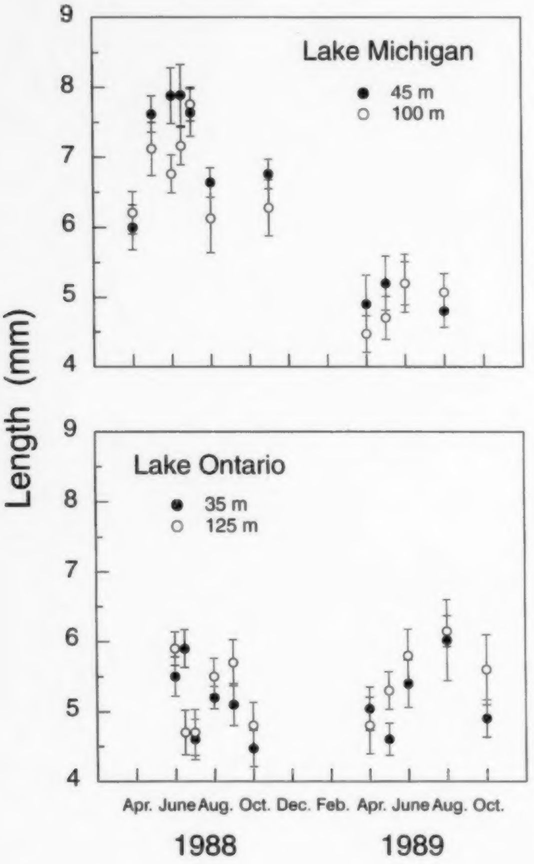
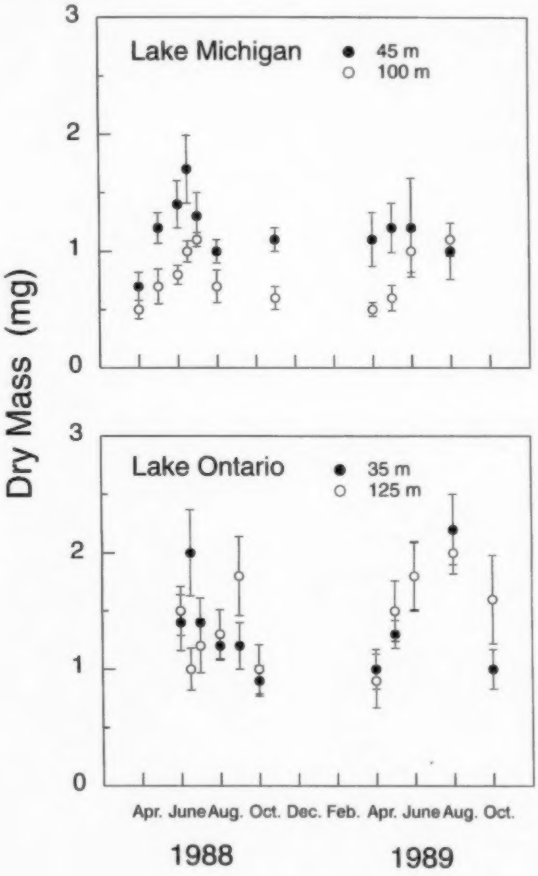


Fig. 2. Mean dry masses of *Diporeia* from Lake Michigan and Lake Ontario that were examined for total lipid and lipid classes. Error bars are SEs.



1–2 mm class) were determined from preserved samples collected at the shallower depths on dates indicated in Table 1.

Additional samples were taken at each site for measurement of total lipid and lipid classes. These unpreserved samples were transported at or below ambient temperature (4–8°C) to Ann Arbor, Mich., as soon as possible after collection (1–3 days). In the laboratory, juvenile *Diporeia* 4–8 mm long were selected at random. Juveniles were selected to eliminate variation in lipid content that occurs between adults. For example, in one study total lipid of dry mass averaged 23% in female and 9% in male *Diporeia* (Quigely et al. 1989). Selected *Diporeia* were rinsed with distilled water, blotted dry, measured with a digitizing system (Quigley and Lang 1989), and placed in miniature test tubes (6 × 50 mm) for drying (under N_2, at 50°C) and lipid extraction (Folch et al. 1957; Gardner et al. 1985*b*). For every lipid sample collection date, length, dry mass, and total lipid were determined on 8–13 individuals from each site in each lake, while lipid classes were determined on only a portion of these individuals ($n = 2$–6). Total lipids were extracted using chloroform:methanol (2:1, v/v), and quantified gravimetrically. Lipid classes were analyzed by thin layer chromatography with flame ionization detection (TLC-FID). Lipid classes were separated by spotting a portion of the lipid extract onto silica-coated Chromarods-SII or -SIII (RSS Inc.) and sequentially developing them in solvent systems of increasing polarity. Chromarods were scanned after each group separation in an Iatroscan Mark IV (Iatron Labs, Tokyo, Japan). A mixed lipid standard, containing one compound from each of the following lipid classes: hydrocarbon, sterol ester, triacylglycerol, free fatty acid, alcohol (aliphatic), sterol (alicyclic), and phospholipid, was used for TLC-FID calibration and quantification. Calibration curves were determined over a range of 0.15–30 μg for each standard compound (Parrish 1986, 1987).

Statistical differences were determined using a two-tailed *t*-test (SYSTAT, Inc. 1992). Variances were tested for homogeneity using the *F*-statistic.

Results

Mean lengths of *Diporeia* used for lipid analysis were 5.2 ± 0.1 (mean ± SE) and 5.3 ± 0.1 mm from both depths in Lake Ontario in 1988 and 1989, respectively. In Lake Michigan, the mean lengths of amphipods from both depths were 7.3 ± 0.1 mm in 1988, and 4.9 ± 0.1 mm in 1989 (Fig. 1, Table 2). Mean dry masses were 1.2 ± 0.1 mg in 1988 and 1.0 ± 0.1 mg in 1989 for *Diporeia* from Lake Michigan. In Lake Ontario, *Diporeia* mean dry masses were 1.3 ± 0.1 mg and

Table 2. Summary of means (± SEs) for 1988 and 1989 *Diporeia* from Lakes Michigan and Ontario, with *n* given in parentheses.

	Density (individuals·m^{-2})	Biomass (g·m^{-2})	Dry mass (mg)	Length (mm)	Total lipid (% of DM)	TG (% of TL)
			1988			
Lake Michigan						
45 m	7 831 ± 521 (7)	6.0±0.4	1.4±0.1 (48)	7.6±0.2	28.2±1.0	82.5±2.5 (14)
100 m	5 311 ± 243 (7)	1.9±0.1	0.9±0.1 (39)	7.0±0.2	20.4±0.9	66.0±3.4 (14)
Combined	6 572 ± 445 (14)	4.0±0.6	1.2±0.1 (87)	7.3±0.1	24.7±0.8	73.5±2.6 (28)
Lake Ontario						
35 m	7 378 ± 431 (6)	3.0±0.1	1.3±0.1 (53)	5.1±0.1	35.7±1.1	81.0±2.7 (20)
125 m	3 185 ± 365 (3)	0.9±0.1	1.3±0.1 (57)	5.3±0.1	32.4±1.2	72.3±3.6 (20)
Combined	5 980 ± 768 (9)	2.0±0.3	1.3±0.1 (110)	5.2±0.1	34.0±0.8	76.7±2.3 (40)
			1989			
Lake Michigan						
45 m	9 787 ± 789 (4)	11.4±0.9	1.1±0.1 (40)	5.0±0.2	25.7±1.2	76.0±2.9 (18)
100 m	5 176 ± 410 (4)	4.2±0.5	0.8±0.1 (41)	4.9±0.2	17.9±1.2	56.4±5.4 (16)
Combined	7 482 ± 964 (8)	7.8±1.5	1.0±0.1 (81)	4.9±0.1	21.7±1.0	67.4±3.3 (34)
Lake Ontario						
35 m	10 006 ± 1202 (3)	3.1±0.5	1.4±0.1 (39)	5.2±0.1	37.4±1.1	84.2±1.2 (15)
125 m	2 971 ± 480 (3)	1.1±0.2	1.4±0.1 (39)	5.4±0.1	32.5±1.3	76.9±3.4 (16)
Combined	6 488 ± 1676 (6)	2.1±0.5	1.4±0.1 (78)	5.3±0.1	34.9±1.2	80.6±1.9 (31)

Note: The *n* given for density is the same for biomass and the *n* for dry mass (DM) is the same for length and total lipid (TL). The triacylglycerol (TG) *n* is given separately. Density and biomass means were determined from the same months as in Fig. 4. To compare a similar season, the annual means for DM, length, TL, and TG were determined from Lake Ontario collection dates from June through the middle of September and from Lake Michigan dates from June through late August in 1988. In 1989 the means were determined from the collection dates from April through August in both lakes (see Table 1 for exact dates).

1.4 ± 0.1 mg in 1988 and 1989, respectively (Fig. 2, Table 2). In Lake Michigan, *Diporeia* from the 45-m depth differed ($P < 0.05$) in mean length and mass from individuals collected at 100 m in 1988 (Table 2). In 1989, *Diporeia* from 45 m were heavier on average ($P < 0.05$) than those from 100 m; however, the mean lengths from both depths were not different ($P > 0.05$). In contrast, regardless of year, the Lake Ontario *Diporeia* from 35 and 125-m depths were similar ($P > 0.05$) in mean mass or length (Table 2).

Length–mass relationships of *Diporeia* were determined for each lake, year and depth combination (Table 3). The best relationship was $\ln(DM) = a + b\ln(L)$, where DM is amphipod dry mass (milligrams) and L is length (millimetres). All relationships were significant ($P < 0.001$), and there were no significant differences among the variances of the residuals (Cochran's test: Beyer 1991) or slopes (GLM test for equality of slopes) of the eight regressions. However, based on a common slope of 2.602, there were significant differences among the intercepts (analysis of covariance; Hald 1967; SYSTAT, Inc. 1992). A post-hoc Tukey HSD multiple comparison test revealed that five of the intercepts were similar while three were significantly different from each other and also different from the other five. The order of mass per given length of these three was 100-m Lake Michigan 1988 < 45-m Lake Michigan 1988 < 100-m Lake Michigan 1989. Dry masses of all of the Lake Ontario *Diporeia* and those from Lake Michigan at 45 m in 1989 were not significantly different from each other but had the greatest dry masses for a given length.

Mean total lipid levels of *Diporeia* (as percentage of dry mass) from Lake Ontario were higher ($P < 0.05$) in 1988 and 1989 than in Lake Michigan individuals from both years (Table 2). This difference between lakes was especially apparent at the deep sites in August 1988 when *Diporeia* total lipid levels were over twice as high in Lake Ontario than in Lake Michigan (Fig. 3). Triacylglycerol (TG), the energy reserve lipid, was the most abundant lipid class in *Diporeia* from both lakes; it paralleled seasonal changes in total lipid. The most pronounced seasonal changes in TG occurred at the 125-m site in Lake Ontario in 1988 (Fig. 3). The largest TG, and therefore total lipid increase, occurred in August and September of that year; however, this late summer TG peak was not apparent in 1989. In Lake Michigan, the largest TG levels occurred during summer at both sites, in both 1988 and 1989 (Fig. 3). Phospholipid (PL; structural cell membrane lipid) constituted between 2 and 53% of the total lipids present in *Diporeia*. A high percentage of PL occurred when storage lipids (TG) were low. Other lipid classes that were analyzed occurred at relatively low levels and were combined; these classes include hydrocarbons, methyl esters, sterol esters, sterols, and the operationally defined acetone mobile polar lipids (including monoacylglycerols, glycolipids, and chlorophyll pigments; Parrish 1986).

Diporeia densities (based on >2 mm amphipods) were similar in both lakes at the shallow depths; at the deeper sites, *Diporeia* densities were higher in Lake Michigan than in Lake Ontario (Fig. 4A, Table 2). Nevertheless, *Diporeia* biomass in Lake Michigan was higher than in Lake Ontario at the shallow depth in 1988, and at both depths in 1989 (Fig. 4B, Table 2). This was due principally to the frequency of larger animals in Lake Michigan (Fig. 5).

Fig. 3. *Diporeia* mean percent total lipid and lipid classes in Lake Ontario from the 35- and 125-m sites and in Lake Michigan *Diporeia* from the 45- and 100-m sites. Error bars are SEs of the mean total lipid. Lipid classes are represented as triacylglycerols (TG), phospholipids (PL), and other lipids (OL). *Diporeia* were analyzed in both early and late June 1988. See Table 1 for exact dates.

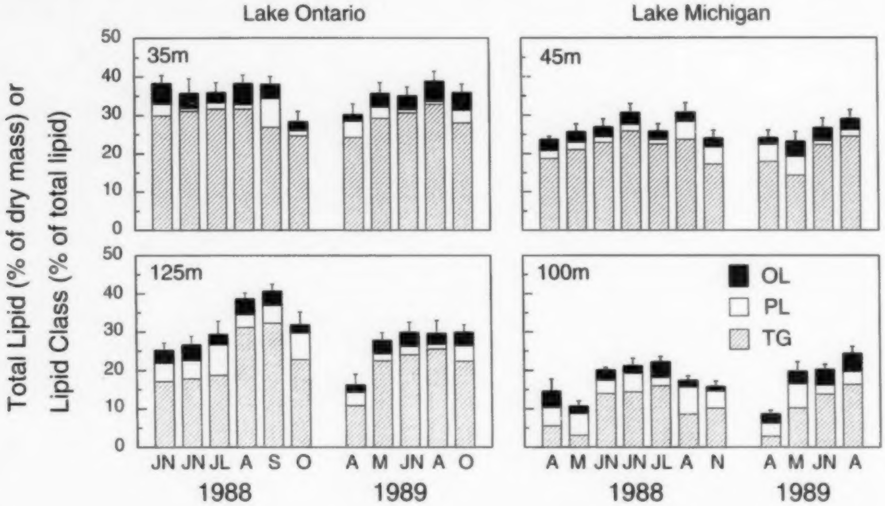


Table 3. Summary of regression analyses for *Diporeia* dry mass (mg) versus length (mm).

Lake	Year	Depth (m)	*a*	*b*	R^2	*n*
Michigan	1988	45	–4.946	2.556	0.831	77
	1989	45	–3.695	2.300	0.892	40
Ontario	1988	35	–3.974	2.544	0.760	65
	1989	35	–3.625	2.371	0.717	49
Michigan	1988	100	–5.071	2.471	0.688	67
	1989	100	–4.704	2.752	0.765	41
Ontario	1988	125	–4.387	2.718	0.789	68
	1989	125	–4.843	3.005	0.861	48

Note: Both variables were first log transformed using the model: $\ln(DM) = a + b \ln(L)$, where DM is dry mass and *L* is length. All slopes (*b*) and intercepts (*a*) are significantly different from zero ($P < 0.001$). All regressions are significant ($P < 0.001$).

Discussion

Lakes Michigan and Ontario are different in many limnological respects, and *Diporeia* populations reflect these differences. The higher total lipid levels of Lake Ontario *Diporeia* relative to those in Lake Michigan regardless of depth, suggests that food inputs are more abundant in Lake Ontario even in the profundal region. This finding was unexpected given low SiO_2 concentrations that could perhaps reduce the spring diatom bloom in Lake Ontario as compared with Lake Michigan (Sly and Christie 1992; Johengen et al. 1994). However, Lake Ontario is more productive than Lake Michigan, and the majority of spring phytoplankton are large diatoms such as *Melosira* spp. that remain abundant until early June and July at the 35- and 125-m sites, respectively (Johannsson et al. 1985; Gray 1987; Johengen et al. 1994). Lipids in diatoms and other phytoplankton increase under stress (i.e., nutrient limitation) (Roessler 1988; Shifrin and Chisholm 1981). SiO_2 limitation may induce greater lipid production in diatoms in Lake Ontario than in Lake Michigan. Upon their settling to the benthos, lipid-rich diatoms would be a direct high energy food source to *Diporeia*. In addition, Lake Ontario is dominated by small zooplankton such as rotifers and cyclopoid copepods (Johannsson et al. 1991; Mazumder et al. 1992) that may not consume large diatoms, thus allowing a high proportion of cells to settle to the bottom and become available to benthic organisms.

During most months the 3–5 mm amphipods were more abundant in Lake Ontario while 6–11 mm individuals were more abundant in Lake Michigan (Fig. 5). The predominance of smaller individuals in Lake Ontario may reflect differences in fish predation rather than food limitation because lipid reserves in juvenile *Diporeia* were relatively high. In Lake Ontario, significant correlations suggest that slimy sculpin (*Cottus cognatus*) suppress *Diporeia* biomass (Sly and Christie 1992). In addition, large juvenile (>5 mm) and adult *Diporeia* are most commonly found in deepwater sculpin (*Myoxocephalus thompsoni*) guts (Evans et al. 1990). This implies that fish predation will reduce the larger size-classes of *Diporeia*. Lake whitefish (*Coregonus clupeaformis*) also prey on *Diporeia*, and their relative abundances have increased recently in eastern Lake Ontario (Christie et al. 1987; Casselman and Scott 1992). In Lake Michigan, benthic predation is dominated by adult bloater (*Coregonus hoyi*) that appear to feed in a narrower prey size range (>4 to <8 mm) than whitefish or sculpin. These gape-limited foragers may permit the largest *Diporeia* to escape predation in Lake Michigan (McDonald et al. 1990).

Differences in *Diporeia* areal biomass and lipid levels may reflect different lower food-web interactions in Lake Michigan and Lake Ontario. The Lake Michigan spring diatom bloom is coupled to benthic organisms, especially *Diporeia*,

Fig. 4. Mean *Diporeia* densities (A) and biomasses (B) from two depths in lakes Ontario (LO) and Michigan (LM) in 1988 and 1989.

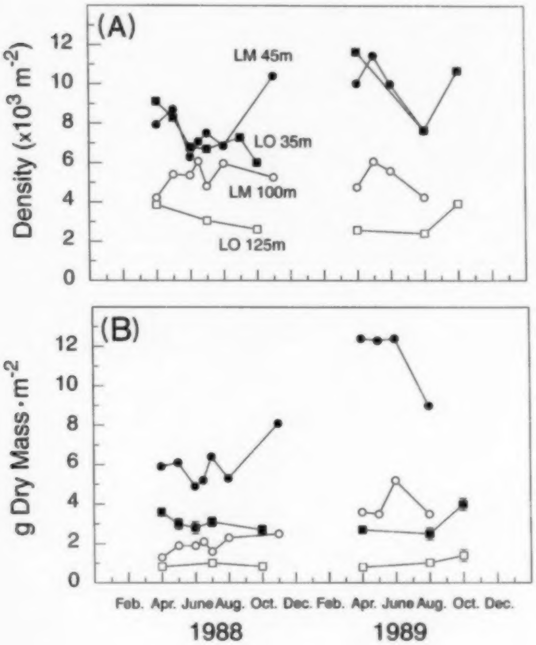
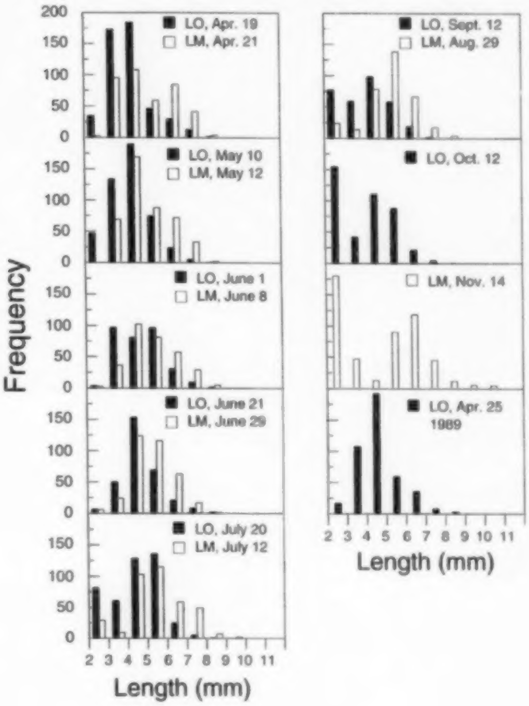


Fig. 5. *Diporeia* size-class frequency distributions from the Lake Ontario (LO) 35-m and Lake Michigan (LM) 45-m sites.



that are in turn preyed upon by fish (Gardner et al. 1990; Fitzgerald and Gardner 1993). However, it appears that *Diporeia* lipid levels have declined slightly in Lake Michigan at the 45-m site from a mean of 33.7 ± 3.3% of dry mass in 1984 to 28.2 ± 1.0% of dry mass in 1988, and then to 25.7 ± 1.2% of dry mass in 1989 (Gardner et al. 1985*a*; Table 2). The relatively low lipid levels in Lake Michigan *Diporeia* suggest that the Lake Michigan spring diatom bloom is not as great as that in Lake Ontario or that the diatoms are being intercepted by zooplankton before they settle to the benthos. Spring NO_3 and SiO_2 uptake rates suggest that diatoms contribute more to total springtime production in Lake Michigan than in Lake Ontario (Johengen et al. 1994). In 1988, mean springtime algal biomasses were 1.5 $g \cdot m^{-3}$ at the 100-m site in Lake Michigan (G.L. Fahnenstiel, Lake Michigan Field Station, NOAA, Great Lakes Environmental Research Laboratory, Muskegon, Mich., unpublished data) and 1.0 $g \cdot m^{-3}$ at the 125-m site in Lake Ontario (O.E. Johannsson, DFO, Great Lakes Laboratory for Fisheries and Aquatic Science, Canada Centre for Inland Waters, Burlington, Ont., unpublished data). It is possible that spring diatoms are being intercepted before they can reach the benthos by hypolimnetic calanoid copepods that are dominant in Lake Michigan. Calanoid copepods are a minor component of the zooplankton community in Lake Ontario (Mazumder et al. 1992; Johannsson et al. 1985). Recently, the calanoid copepods *Diaptomus sicilis* and *Limnocalanus macrurus* have been increasing in numbers in Lake Michigan (G.L. Pernie, NOAA, Great Lakes Environmental Research Laboratory, Ann Arbor, Mich., personal communication) and will probably increase grazing pressure on spring diatoms. Early spring feeding experiments with *D. sicilis* revealed that high feeding rates occurred during under-ice and post-ice phytoplankton blooms in Grand Traverse Bay, Lake Michigan (Vanderploeg et al. 1992*a*). The decline in *Diporeia* lipid levels since 1984 may also suggest that the settling of high quality food to the benthos has been reduced in offshore Lake Michigan. In addition, the longer settling time required for diatoms to reach greater depths would allow for more zooplankton grazing and microbial decomposition that would cause a lower percentage of total diatoms to reach the profundal zone (Eadie et al. 1984). This may explain the greater range in *Diporeia* lipid reserves between the shallow (slope) and deep sites in Lake Michigan than in Lake Ontario (Table 3).

The higher lipid levels of *Diporeia* from Lake Ontario, compared with Lake Michigan *Diporeia*, may indeed reflect differences in the food web of the two lakes. However, the different populations may also have genetic explanations for their modes of food assimilation and lipid storage patterns, as do two sympatric species of amphipods from the Baltic Sea, *Monoporeia affinis* and *Pontoporeia femorata*. Although they dwell in the same sediments, *M. affinis* has a higher respiration and feeding rate than *P. femorata*, and thus has faster lipid accumulation and turnover rates than the more seasonally stable lipid storage pattern of *P. femorata* (Hill et al. 1992). Whereas eight or more species of *Diporeia* may be found in North America, exact species identifications have not been determined in the Great Lakes region (Bousfield

1989). Ideally, further morphological and genetic studies are necessary to clarify species within *Diporeia*.

Finally, lipid storage patterns can reveal a great deal about an organism's life history. For example, many invertebrates need to accumulate a certain percentage of lipids before they can reproduce or metamorphose into adults as is the case for some insects (Hill et al. 1992; Vanderploeg et al. 1992*b*; Cargill et al. 1985). Adult *Diporeia* females have higher lipid levels than males or juveniles (Quigley et al. 1989). Most of the lipids are then transferred from the gravid females to their brooded young to give them an advantage in their first few days of life. In addition, lipid-rich females may produce large broods. In support of this idea, Lake Ontario gravid female *Diporeia* brooded a mean of 29 eggs (R. Dermott, unpublished data) while gravid females from Lake Michigan brooded a mean of 17 eggs (Winnell and White 1984).

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Occurrence and effect of the parasitic isopod, *Lironeca ovalis* (Isopoda: Cymothoidae), on young-of-the-year bluefish, *Pomatomus saltatrix* (Pisces: Pomatomidae)

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Abstract: The bluefish, *Pomatomus saltatrix*, is often a preferred host for infection by the isopod, gill ectoparasite *Lironeca ovalis*. Here we quantify the occurrence of infection by *L. ovalis* on young-of-the-year bluefish in the Hudson River estuary and evaluate whether there is a significant reduction in growth or foraging as a result of infection. Prevalence of *L. ovalis* on bluefish was high (25.4%) but small (<75 mm) and large fish (>175 mm) exhibited significantly lower prevalences than intermediate-sized fish. Parasite size increased with bluefish size, and a significant but small effect of parasitism on the bluefish mass–length relationship was found; parasitized fish weighed 3% less than nonparasitized fish at a given length. Infection did not have a significant effect on the mass of stomach contents at capture. Although there remain many questions regarding the host–parasite relationship between *P. saltatrix* and *L. ovalis*, the physiological cost associated with infection appears to be small and probably does not constitute a serious threat to individual bluefish survival.

Résumé : Le tassergal (*Pomatomus saltatrix*) est souvent un hôte privilégié de l'isopode (*Lironeca ovalis*), ectoparasite des brachies. Nous avons mesuré la fréquence d'infection par le *L. ovalis* chez les jeunes tassergals de l'année, dans l'estuaire du fleuve Hudson, et évalué dans quelle mesure cette infection entraîne une réduction significantive de la croissance ou de la recherche de nourriture. La fréquence du parasite était globalement élevée (25,4%), mais significativement moindre chez les tassergals de taille petite (<75 mm) ou grande (>175 mm) que chez ceux de taille intermédiaire. La taille des parasites augmentait avec celle de l'hôte, et le parasitisme avait un effet significatif, bien que léger, sur le rapport masse–longueur : pour une longueur donnée, les sujets parasités pesaient 3 % de moins que ceux qui ne l'étaient pas. L'infection n'avait pas d'effet significatif sur la masse du contenu stomacal au moment de la capture. De nombreux aspects de la relation hôte–parasite entre le *P. saltatrix* et le *L. ovalis* restent à élucider, mais le coût physiologoique de l'infection semble faible et ne constitue sans doute pas une menace grave pour la survie individuelle des tassergals.
[Traduit par la Rédaction]

Introduction

Lironeca ovalis (Crustacea: Isopoda: Cymothoidae) is a protandrous, hermaphroditic, gill parasite (Brusca 1981; Kabata 1984). The bluefish, *Pomatomus saltatrix* (Pisces: Pomatomidae) is a pelagic teleost that spawns in continental shelf waters along the east coast of the United States (Kendall and Walford 1979). Larval and pelagic juvenile bluefish are transported from offshore spawning grounds to estuarine nursery habitat (Kendall and Walford 1979; Hare and Cowen 1996) where they spend a majority of their first year (Kendall and Walford 1979; McBride and Conover 1991). While in estuaries, young-of-the-year (YOY) bluefish grow rapidly (McBride and Conover 1991; Juanes and Conover 1994). *Lironeca ovalis* frequently parasitize YOY bluefish (Richardson 1905; Fowler 1912; Lindsay and Moran 1976; Sandifer and Kerby 1983) and may affect individual survival by reducing foraging ability and (or) growth.

The physiological effects of cymothoid infection are not clearly understood. Deleterious effects on host finfish have been reported in several studies citing tissue damage; host behavioral changes; decreases in mean weight, size, and growth; and, in some instances, death (Kroger and Guthrie 1972; Sadzikowski and Wallace 1974; Lindsay and Moran 1976; Brusca 1978; Romestand and Trilles 1979; Brusca 1981; Kabata 1984; Moser and Sakanari 1985; Segal 1987). Other research has concluded that individual fish infected with a single parasite were not significantly "less healthy" than nonparasitized fish (Brusca 1981; Ruiz and Madrid 1992; Landau et al. 1995) and, only in cases of multiple infection or stress, would an effect on condition and (or) behavior of hosts be realized (Keys 1928; Lanzing and

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O'Connor 1975; Brusca 1981). Similarly, Weinstein and Heck (1977) suggested that host damage, if any, due to cymothoid infection is minimal.

The objective of this study was to quantify the occurrence and evaluate the effect of infection by *L. ovalis* on YOY bluefish in the Hudson River Estuary. The infection rate (prevalence) relative to bluefish length was elucidated, as was the parasite mass – bluefish length relationship. The effect of parasitism on bluefish growth and feeding was also examined through analysis of the bluefish mass–length relationship and weight of stomach contents.

Materials and methods

Young-of-the-year, spring-spawned bluefish (n = 768) were collected from the lower 40–65 km of the Hudson River. Collections were made biweekly from mid-July to early November in 1989 and 1990 using a beach seine (61 × 3 m with 13 mm mesh wings and a 6 mm mesh bag) set from a boat. Bluefish and isopods were killed and preserved in 10% buffered formalin. In the laboratory, stomachs were emptied, and in 1989, the contents were weighed to the nearest 0.001 g. Bluefish, minus their stomach contents, were wet weighed to the nearest 0.01 g and measured for total length (hereafter length) to the nearest 1 mm. This work was part of a larger studying examining the diet of YOY bluefish (see Juanes et al. 1993).

The number of *L. ovalis* on each bluefish was enumerated. Isopods were then removed from their host and weighed to the nearest 0.001 g. The gills of nonparasitized bluefish were examined for the presence of scars that would imply previous parasitism. None were found.

The relationship between prevalence of the isopod and host size was evaluated using nonparametric regression based on splines (see Schluter 1988; Anderson 1995). The method, as developed by Anderson (1995), estimates relative survival with regard to a quantitative trait based on cross-sectional data (before–after comparisons). Nonparametric regression based on splines was used in this study to estimate the rate of parasitism relative to bluefish length. Rather than a before–after comparison, lengths of parasitized and nonparasitized bluefish were compared. The 95% confidence limits were calculated with 10 bootstrapped replications. This method assumes parasitism is a smoothly changing function of bluefish length, makes no a priori assumption as to a parametric model, does not require grouping of data, and allows calculation of confidence intervals about the function (see Schluter 1988; Anderson 1995).

A randomization procedure was used to evaluate the estimated prevalence relative to bluefish length. In the actual data, 195 out of 768 bluefish were infected with *L. ovalis*. The randomization procedure used the actual lengths of bluefish and randomly classified 195 of these 768 lengths as being parasitized fish. The nonparametric regression procedure was then used to estimate the function of parasitism rate relative to bluefish length based on the randomly generated data set. Randomization was repeated 10 times, and for each bluefish length, the average estimated parasitism rate (if parasitism was a random function of bluefish length) was calculated, as were the 95% confidence limits. If the estimated function based on the actual data fell outside the 95% confidence limits of the estimated function based on random data, then it was concluded that parasitism was not independent of bluefish length (see Sokal and Rohlf 1981).

The relationship between parasite mass – host length was analyzed with linear regression (Sokal and Rohlf 1981) using the multiple general linear hypotheses module of SYSTAT (Wilkinson 1990). Bluefish length and isopod mass were log transformed before calculation of the regression.

To evaluate the effect of parasitism on bluefish growth, the log-transformed bluefish mass–length relationship was analyzed using an ANCOVA model (Sokal and Rohlf 1981). First, data from the 2 years were compared for bluefish between 100 and 150 mm length (n = 484) with the model: log(mass)= $a + b \times$ log(length) + $c \times$ year + $d \times$ log(length) × year. The interaction term was not significant ($p > 0.05$) and the reduced ANCOVA model: log(mass) = $a + b \times$ log(length) + $c \times$ year was calculated. The year term was not significant ($p > 0.05$), and thus, the data from the 2 years were pooled. An ANCOVA model was then used to determine the effect of parasitism on the mass–length relationship. The significance of the interaction term was first determined with the model: log(mass) = $a + b \times$ log(length) + $c \times$ parasitized + $d \times$ log(length) × parasitized. The significance of the parasitism term was then determined with the model: log(mass) = $a + b \times$ log(length) + $c \times$ parasitized. Calculations were made with the multiple general linear hypotheses module of SYSTAT (Wilkinson 1990). In addition, the allometric coefficient b ($W = aL^b$) describing the rate of isopod mass increase relative to bluefish length and bluefish mass increase relative to bluefish length were compared (Sokal and Rohlf 1981).

The effect of parasitism on bluefish feeding was analyzed with an ANCOVA model (Sokal and Rohlf 1981) using the stomach content mass data collected in 1989. Bluefish length and stomach content weight were log transformed; because many bluefish had no prey items in their guts, a log(stomach content mass + 1) transformation was used. The significance of the interaction term was first determined with the model: log(stomach content mass + 1) = $a + b \times$ log(length) + $c \times$ parasitized + $d \times$ log(length) × parasitized. The significance of the parasitism term was then determined with the model: log(stomach content mass + 1) = $a + b \times$ log(length) + $c \times$ parasitized.

Results

Of 768 bluefish collected, 25.4% (195) were infected with a single *L. ovalis*. All isopods were attached to the host either on the anterio-ventral portion of the gill chamber (n = 192) or in the buccal cavity (n = 3). Bluefish were 45–278 mm in length and weighed 0.96–220.26 g. Isopods weighed 0.030–0.880 g.

The estimated function of isopod prevalence in relation to bluefish length was complex with a steep increase at about 75 mm, a broad but varying plateau (~30%) between 75 and 175 mm, and a gradual decrease at sizes greater than 175 mm (Fig. 1). Fewer fish were parasitized at sizes <75 mm and >175 mm than expected if parasitism was a random function of length (Fig. 1).

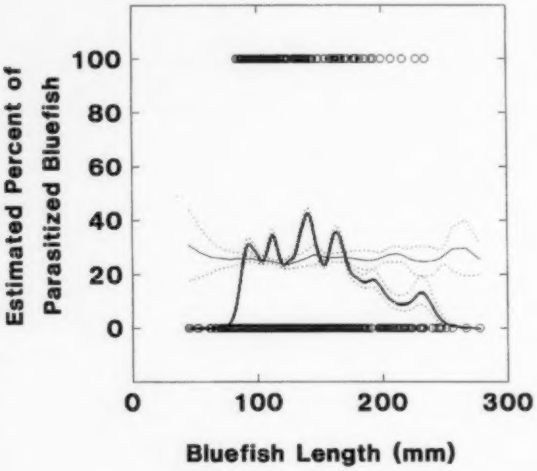
Parasite mass increased with increasing bluefish length (Fig. 2). The linear regression model (log(parasite mass) = $a + b \times$ log(length) was significant ($p < 0.001$, $r^2 = 0.41$) as were both terms of the model ($a = -5.61$, SE = 0.40, $p < 0.001$; $b = 2.29$, SE = 0.19, $p < 0.001$).

Although there was a significant effect of parasitism on the bluefish mass–length relationship, this effect was very small. Parasitized fish weighed only 3% less than nonparasitized fish at a given length (Fig. 3). Using the full ANCOVA model, the interaction term was nonsignificant (Table 1). However, without the interaction term, a significant but small parasite effect was found (Table 1).

Bluefish mass increased more rapidly with bluefish length than did isopod mass. The allometric coefficient b ($W = aL^b$) for the isopod mass – bluefish length relationship ($b = 2.29$, SE = 0.19) differed from that for the bluefish mass–length relationship ($b = 3.15$, SE = 0.01) ($\mathrm{MSD}_{\alpha=0.05} = 0.383$).

Parasitism did not affect stomach content mass of bluefish. Using the full ANCOVA model with length as a covariate, the interaction term was not significant (Table 2). Without the

Fig. 1. Nonparametric regression estimates of prevalence relative to bluefish length. The estimated function based on the actual data is denoted by the thick line, and the estimated function based on the randomly generated data is denoted by the thin line. 95% confidence limits around each function are shown as broken lines. Individual lengths for all bluefish, both parasitized and nonparasitized, are denoted by open circles.



interaction term, the parasite effect was also nonsignificant (Table 2).

Discussion

During estuarine residency, young bluefish are often preferred hosts for cymothoid infection (Thomas et al. 1974; Lindsay and Moran 1976; Brusca 1981; Rokicki 1985). In this study, overall prevalence of *L. ovalis* on YOY bluefish from the Hudson River Estuary was high (25.4%). This generally high level of infection, as compared with other species (Table 3), suggests that the bluefish is a uniquely suitable host for *L. ovalis*. The tendency of bluefish to school (Van der Elst 1976), combined with an estuarine-dependent early life history (McBride and Conover 1991) and one of the highest specific growth rates reported for temperate fishes (1–2 mm · day^{-1}, McBride and Conover 1991; Juanes and Conover 1994), may partially explain the relationship.

The decreasing prevalences at larger host sizes that we observed is consistent with other reports of infection on larger juveniles and offshore adults (Anderson 1970; Sadzikowski and Wallace 1974; Thomas et al. 1974; Lanzing and O'Connor 1975; Brusca 1981). The rapid increase in prevalence at smaller sizes has not been reported previously. Future studies should examine the ecological causes of size dependency in the host–parasite relationship.

A positive, significant host–parasite size relationship was found in this study (Fig. 2) and in other studies on bluefish (Landau et al. 1995), lutjanids (Weinstein and Heck 1977), *Citharichthys* spp. (Robinson 1982), and carangids (Menzies et al. 1955). Despite the significance of this relationship, the correlation coefficient ($r^2 = 0.41$) indicates substantial variability that may reflect differences in host size at the time of infection.

Fig. 2. Isopod mass – bluefish length relationship with the fitted allometric function ($W = aL^b$) based on the linear regression of the log-transformed data ($\log(W) = \log(a) + b \log(L)$).

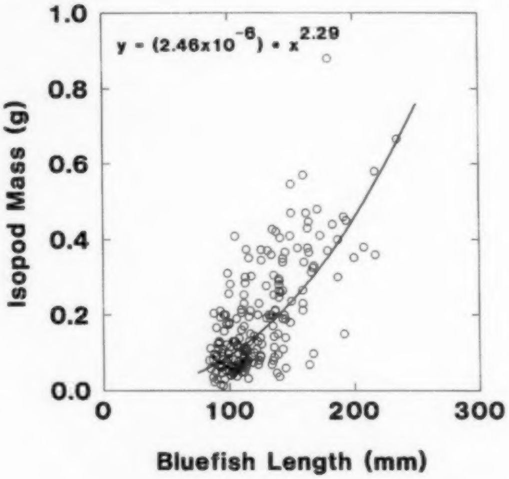
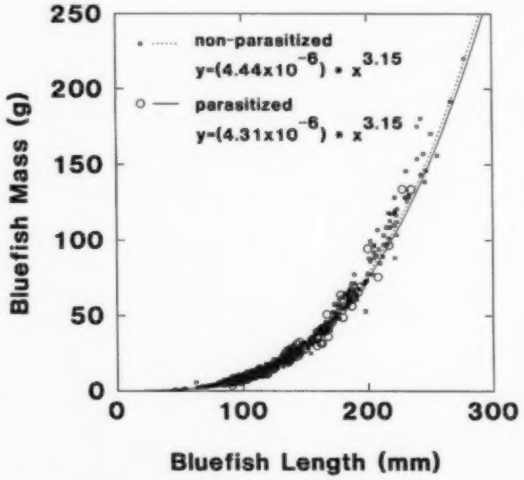


Fig. 3. Mass–length relationship for both parasitized and nonparasitized bluefish. The fitted allometric functions ($W = aL^b$) based on the reduced ANCOVA model for both parasitized and nonparasitized bluefish are also shown ($\log(W) = (\log(a) \pm \text{parasitism effect}) + b \log(L)$.



Keys (1928) and Brusca (1981) suggested that only in stressful environmental situations will a deleterious, parasite-induced influence on host vitality be realized. Hence, one might expect evidence of a serious negative effect on condition in the polluted environment of the lower Hudson River (Limburg et al. 1986). We found no evidence of a serious effect due to parasitism. Although there was a significant effect of parasitism on the host mass–length relationship (Table 2), infected fish weighed only 3% less than nonparasitized individuals for a given length (Fig. 3).

Table 1. Summary of the ANCOVA analysis of the effect of parasitism on the bluefish mass–length relationship.

(A) Full ANCOVA model (log(mass) = $a + b \times$ log(length) $+ c \times$ parasitized $+ d \times$ log(length) × parasitized).

Source	df	MS	*F*	*p*
Log(length)	1	61.571	29 142.979	<0.001
Parasitized	1	0.001	0.545	ns
Log(length) × parasitized	1	0.001	0.317	ns
Error	764	0.002		

(B) Reduced ANCOVA model (log(mass) = $a + b \times$ log(length) $+ c \times$ parasitized).

Source	df	MS	*F*	*p*
Log(length)	1	122.430	58 001.140	<0.001
Parasitized	1	0.027	12.649	<0.001
Error	765	0.002		

Table 2. Summary of the ANCOVA analysis of the effect of parasitism on the bluefish stomach content mass – bluefish length relationship.

(A) Full ANCOVA model (log(stomach content mass + 1) = $a + b \times$ log(length) + $c \times$ parasitized $+ d \times$ log(length) × parasitized).

Source	df	MS	*F*	*p*
Log(length)	1	6.038	120.246	<0.001
Parasitized	1	0.004	0.769	ns
Log(length) × parasitized	1	0.006	0.727	ns
Error	367	0.050		

(B) Reduced ANCOVA model log(stomach content mass + 1) = $a + b \times$ log(length) $+ c \times$ parasitized).

Source	df	MS	*F*	*p*
Log(length)	1	11.607	231.685	<0.001
Parasitized	1	0.053	1.061	ns
Error	368	0.050		

Other studies have found that parasitic infection may reduce or interfere with the ability of the host to feed (Menzies et al. 1955; Weinstein and Heck 1977). However, our data indicate that infection by *L. ovalis* had little effect on the mass of bluefish stomach contents. While infected bluefish may weigh slightly less for a given length, infection does not appear to impair feeding. This result implies that the difference in the infected versus noninfected bluefish mass–length relationships was not due to differential feeding ability or behavioral changes affecting foraging success; the basis of this difference remains to be resolved. We conclude that infection by *L. ovalis* probably does not constitute a serious threat to YOY bluefish survival.

According to Nikolsky (1978), teleost–parasite interactions are defined in two ways: those where parasites benefit from host survival and those where parasite fitness is enhanced by death of the host. In relationships where survival of the host fish is important, parasitic species may consume tissues or fluids in a manner that does not cause serious harm to the host (Nikolsky 1978). Several aspects of isopod life history support the concept that cymothoids may parasitize in a manner that does not constitute a serious threat to host well-being. In the case of *Lironeca* spp., swimming ability is vestigial in adults (Menzies et al. 1955; Brusca 1978; Keusink 1979; Sandifer and Kerby 1983). Thus, it may be critical for a young, mobile isopod to attach to a host and then mature as the host grows. *Lironeca* spp. also exhibit self-regulating behavior regarding the maximum number of individual parasites allocated to any one host (Keusink 1979). Finally, cymothoids produce a small number of eggs per adult female (approx. 300–400). This combination of life-history traits may result in cymothoids being adapted to semi-enclosed habitats where host density is high (Brusca 1981; Rokicki 1985) and where continued host survival is beneficial to the parasite.

Table 3. Summary of *Lironeca ovalis* prevalence for several teleost species, including *Pomatomus saltatrix*, in nearshore habitats of the Middle Atlantic Bight.

Species	Study site	Prevalence (%)	Reference
Pomatomus saltatrix	Hudson River	25.0	This study
P. saltatrix	Great South Bay	7–16.0	R.E. Marks (personal observation)
P. saltatrix	Great Bay	6.0	R.E. Marks (personal observation)
P. saltatrix	Delaware River	20.0	Lindsay and Moran (1976)
P. saltatrix	Sandy Hook Bay	10.0	Breder (1925)
P. saltatrix	Great Bay	2–38.0	Thomas et al. (1974)
P. saltatrix	Jersey Shore	22.0	Landau et al. (1995)
Morone saxatilis	Delaware River	2.0	Lindsay and Moran (1976)
M. saxatilis	Long Island	2.0	Alperin (1966)
M. saxatilis	Chesapeake Bay	7.0	B.H. Young (personal communication)[a]
M. saxatilis	Long Island Sound	12.0	B.H. Young (personal communication)[a]
Morone americana	Delaware River	2.0	Sadzikowski and Wallace (1974)
M. americana	Delaware River	4.0	Lindsay and Moran (1976)
Bairdiella chrysura	Delaware River	6.0	Lindsay and Moran (1976)
Cynoscion regalis	Delaware River	3.0	Lindsay and Moran (1976)
Leiostomus xanthurus	Delaware River	0.4	Lindsay and Moran (1976)
Micropogonius undulatus	Delaware River	0	Lindsay and Moran (1976)
Menticirrhus saxatilis	Delaware River	0	Lindsay and Moran (1976)
Pogonias cromis	Delaware River	0	Lindsay and Moran (1976)

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Stable isotope analysis of nutrient pathways leading to Atlantic salmon[1]

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Abstract: The relative contribution of terrestrial and aquatic primary energy sources in food webs along a stream continuum in the Miramichi River system, New Brunswick, was investigated through the use of stable carbon and nitrogen isotope ratios. In sites where these primary energy sources were isotopically distinct, quantitative mixing models were used to identify the relative importance of allochthonous carbon in the diets of wild juvenile Atlantic salmon (*Salmo salar*) and other resident stream fishes. The $\delta^{13}C$ data of the stream fauna ranged from −34‰ to −20‰, suggesting variable assimilation of allochthonous and autochthonous carbon sources at the four study sites. Results from the mixing model were congruent with the stream continuum hypothesis, indicating that fishes in the headwaters of Catamaran Brook were more dependent (>85%) on allochthonous carbon sources than those in sites located downstream (36–52%). Stable nitrogen isotope distributions successfully described food web structure in this study, suggesting at least 2.5–3.5 trophic levels in these lotic ecosystems. Stable isotope ratios of carbon were particularly useful for illustrating seasonal changes in food resources of recently emerged age 0+ salmon as maternally derived marine carbon was subsequently diluted by freshwater carbon over the growing season.

Résumé : Pour étudier la contribution relative des sources d'énergie primaire dans des réseaux trophiques aquatiques et terrestres du continuum d'un cours d'eau du réseau hydrographique de la rivière Miramichi, au Nouveau-Brunswick, on a mesuré les rapports d'isotopes stables du carbone et de l'azote. Pour les lieux où les sources d'énergie primaire se distinguaient par leur profil isotopique, on a utilisé des modèles de mélange quantitatifs pour déterminer l'importance relative du carbone allochtone dans l'alimentation du saumon de l'Atlantique (*Salmo salar*) juvénile sauvage et d'autres poissons vivant dans le cours d'eau. Les valeurs de $\delta^{13}C$ mesurées chez la faune du cours d'eau allaient de −34‰ à −20‰, ce qui laisse entrevoir une assimilation variable du carbone allochtone et du carbone autochtone dans les quatre lieux étudiés. Les résultats obtenus au moyen du modèle de mélange sont compatibles avec l'hypothèse du continuum, ce qui signifie que les poissons du cours supérieur du ruisseau Catamaran dépendent davantage (>85%) des sources de carbone allochtone que les poissons des parties plus en aval du cours d'eau (36–52%). Les distributions d'isotopes de l'azote stables donnent une idée représentative, dans le contexte de cette étude, de la structure des réseaux trophiques et indiquent qu'il y aurait 2,5–3,5 niveaux trophiques dans ces écosystèmes lotiques. Les rapports entre les isotopes stables du carbone sont particulièrement utiles pour décrire les changements saisonniers des ressources alimentaires des saumons d'âge 0+ d'éclosion récente car, durant la saison de croissance, le carbone maternel provenant du milieu marin est diluée par le carbone provenant des eaux douces.
[Traduit par la Rédaction]

Introduction

According to Vannote et al. (1980), stream food webs are based on a gradient of terrestrial (i.e., allochthonous) and aquatic (i.e., autochthonous) primary energy sources from headwaters to river mouth. However, the actual dependence of aquatic communities on these energy sources has rarely been measured and is not always clear (Bird and Kaushik 1984). Stable isotope analysis (SIA) is one way to assess the relative importance of allochthonous and autochthonous inputs in streams. Initially used by earth scientists to track the geochemical cycling of elements, the SIA technique is now finding wide application in the biological sciences where carbon, nitrogen, and sulfur have proved most useful in analyzing food web relations (see reviews by Fry and Sherr 1984; Peterson and Fry 1987; Rundel et al. 1989; Lajtha and Michener 1994). Stable isotopes of carbon, ^{13}C and ^{12}C, have been used most often (Rounick and Winterbourn 1986). Their ratios allow the identification of important primary food sources because they are fixed during photosynthesis and are passed along food chains relatively unchanged (DeNiro and Epstein 1978).

Freshwater streams provide favourable conditions for tracing carbon pathways, as terrestrial and aquatic plants often differ in their stable isotopic compositions (Rau 1980; Rounick et al. 1982). Carbon fixed by terrestrial C_3 plants in temperate regions has a characteristic $^{13}C/^{12}C$ ratio (expressed as $\delta^{13}C$, a parts per thousand difference from a standard reference material) of approximately −28‰ (O'Leary 1988). Aquatic plants exhibit a much wider range in $\delta^{13}C$ (−50‰ to −10‰) relative to terrestrial plants, reflecting site-specific

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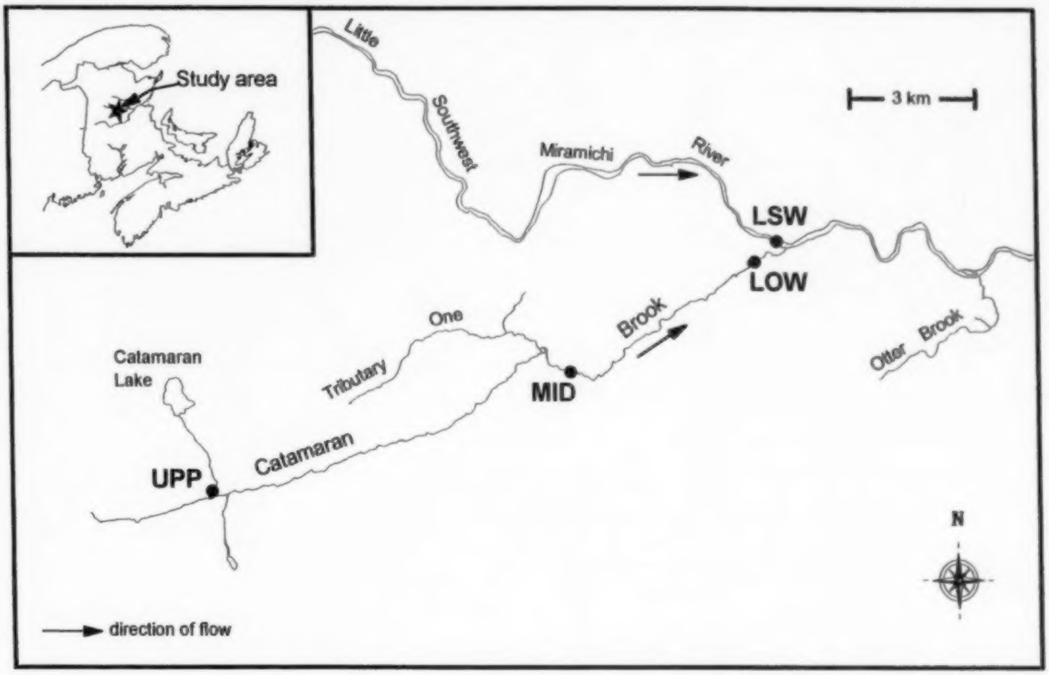
Fig. 1. Map of the study area in New Brunswick, eastern Canada, showing sampling locations in Catamaran Brook (UPP, MID, and LOW) and in the Little Southwest Miramichi River (LSW).



and species-specific factors (Osmond et al. 1981; Farquhar et al. 1989). Recent studies using SIA in the investigation of stream food webs include Bunn et al. (1989), Hesslein et al. (1991), Rosenfeld and Roff (1992), Forsberg et al. (1993), and references therein. Most studies tend to be qualitative; only Junger and Planas (1994) made an attempt to quantify the relative importance of terrestrial and aquatic carbon sources to the stream food web.

We used SIA in an attempt to determine the important primary sources of organic carbon utilized by wild juvenile Atlantic salmon (*Salmo salar*) in Catamaran Brook, New Brunswick. To our knowledge, juvenile Atlantic salmon have not been previously considered in quantitative descriptions of allochthonous–autochthonous carbon dependence (Waters 1993); nor has any prior investigation reported on the stable isotopic composition of food webs in salmon streams of Atlantic Canada. To do this, we measured $^{13}C/^{12}C$ ratios in fish, benthic invertebrates, and terrestrial and aquatic plants. In sites where terrestrial and aquatic plants were shown to be isotopically distinct, quantitative isotopic mixing models (Fry and Sherr 1984) were applied to measure the relative contribution of allochthonous carbon to the fish community. We also analyzed the $^{15}N/^{14}N$ ratios of plants and animals, as an added descriptor of stream food web structure. Nitrogen isotope ratios are enriched by $\approx 3‰$ at each level in the food chain (Minagawa and Wada 1984), thus making them useful indicators of trophic status. Because Catamaran Brook is the site of a long-term scientific study designed to monitor the effects of forestry practices on Atlantic salmon and their instream habitats (Cunjak et al. 1990), a subsidiary aim of our work was to provide baseline stable isotope data against which post-deforestation conditions could be judged. SIA has been used successfully to demonstrate some effects of clearcutting in some New Zealand catchments several years after logging (Rounick et al. 1982; Winterbourn and Rounick 1985; Rounick and Winterbourn 1986).

Methods

Study area

Samples for SIA were collected during 1992 and 1993 in Catamaran Brook (46°52.7′N, 66°06.0′W), a third-order stream in central New Brunswick. Major collection dates included 18–27 May, 2–9 July, 14–19 August, and 8–15 October. Three sites were selected at approximately 18 km (UPP), 8 km (MID), and 1 km (LOW) upstream of the mouth of Catamaran Brook (Fig. 1). An additional site was chosen at the Little Southwest Miramichi River (LSW) about 50 m upstream of the confluence with Catamaran Brook. Morphometric and environmental variables characterizing the study sites are presented in Table 1. A comprehensive description of the physical, chemical, and biological conditions of the brook and its drainage basin is provided in Cunjak et al. (1993).

Collection and preparation of samples

To determine the isotopic signature of the allochthonous carbon source, leaf litter samples were hand-picked from the lower reach of Catamaran Brook (LOW) and sorted to represent common riparian tree species. For site-specific autochthonous carbon labels, the filamentous chlorophyte, *Cladophora* sp., was used because it was available in sufficient quantity at all sites and to avoid the previously reported difficulty of separating terrestrially derived detritus from algae in rock scrapings (Winterbourn et al. 1984; Rounick and Hicks

Table 1. Environmental and morphometric characteristics of the four study sites during the 1992–1993 sampling period.

	Upper Reach (UPP)	Catamaran Brook Middle Reach (MID)	Lower Reach (LOW)	Little Southwest Miramichi River (LSW)
Catchment area (km^2)	12	25	52	1200
Altitude (m)	250	155	75	70
Slope (%)	3.6	1.1	0.6	0.4
Width (m)	3.9	6.9	9.3	90
Mean depth (m)*	0.26	0.28	0.40	0.68
Mean velocity (m·s^{-1})*	0.10	0.35	0.32	0.76
Discharge (m^3·s^{-1})*	0.07	0.68	1.20	17.4
Temperature (°C)*	19	17	20	23
pH*	7.5	7.1	7.0	6.9
Conductivity (μS·cm^{-1})*	59	48	44	25

*Values are means measured during the 1992–1993 sampling period.

1985; Junger and Planas 1994). Other aquatic plants, *Potamogeton* sp. and *Fontinalis* sp., were also collected for determination of their site-specific isotopic signatures. Stream water was sampled at each site for dissolved inorganic carbon (DIC) $\delta^{13}C$ values. Each water sample was sealed in airtight 500-mL glass bottles, preserved with 1 mL of mercuric chloride ($HgCl_2$) solution, and refrigerated until isotope analysis was performed.

Aquatic invertebrates representing major functional feeding groups were identified and used in the analysis: scraper–grazers, *Glossosoma* (Trichoptera: Glossosomatidae) and *Ephemerella* (Ephemeroptera: Ephemerellidae); shredder, *Pteronarcys* (Plecoptera: Pteronarcidae); collector-filterers, *Simulium* (Diptera: Simuliidae) and *Hydropsyche* (Trichoptera: Hydropsychidae); and predator, *Isogenoides* (Plecoptera: Perlodidae). Each sample consisted of enough individuals (up to 10) to provide the minimum of 10 mg (dry weight) needed for isotope analysis. Sections of muscle tissue from behind the dorsal fin and above the lateral line were collected from age 0+ (fork length (FL) 3–5 cm), age 1+ (FL 6–9 cm), and age 2+ Atlantic salmon (FL 10–13 cm), brook trout (*Salvelinus fontinalis*) (FL 8–18 cm), and blacknose dace (*Rhinichthys atratulus*) (FL 5–8 cm).

All samples of plant and animal material were frozen in the field and then freeze-dried in the laboratory. After drying, samples were ground to a powder, and stored in small, airtight, precombusted, glass vials prior to isotope analysis.

Isotope analysis

For SIA, organic materials were converted to gas in either sealed Pyrex (for CO_2) or quartz (for N_2) tubes following the combustion methods described by Boutton et al. (1983) for carbon, and Kendall and Grim (1990) for nitrogen. Extraction of CO_2 from stream water was accomplished by cryogenic separation in an evacuated, closed-line system, following mixture with 2 mL phosphoric acid (H_3PO_4). Analyses were done in the Environmental Isotope Laboratory (Department of Earth Sciences, University of Waterloo, Waterloo, Ont.) using a VG Micromass 903E triple-collector isotope-ratio mass spectrometer. Isotope ratios are expressed as parts per thousand, or "per mil," differences (‰) from a standard reference material:

$$\delta X = \left(\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}}\right) \times 1000$$

where X is ^{13}C or ^{15}N, R is the corresponding ratio, $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and δ is the measure of the ratio of heavy to light isotopes in a sample. Positive, or less negative, δ values denote "enrichment" or increases in the amount of the heavy isotope components. Conversely, decreases in the amount of the heavy isotope indicate "depletion." Standard reference materials, set at arbitrary values of 0‰, are carbon in the Pee Dee limestone formation (Craig 1957) and nitrogen gas in the atmosphere (Mariotti 1983). Precision for the analyses was better than ±0.2‰ for carbon and ±0.5‰ for nitrogen.

Table 2. Stable carbon isotope ratios ($\delta^{13}C$) of decomposing leaf litter from eight of the most common riparian tree species in the Catamaran Brook basin.

Species	$\delta^{13}C$ (‰)
American beech (*Fagus grandifoila*)	–28.6
Speckled alder (*Alnus rugosa*)	–29.5
White birch (*Betula papyrifera*)	–29.5
Yellow birch (*Betula lutea*)	–29.6
Sugar maple (*Acer saccharum*)	–29.8
Red spruce (*Picea glauca*)	–30.6
Balsam fir (*Abies balsamea*)	–30.8
Eastern white cedar (*Thuja occidentalis*)	-31.5

Note: Values are means of duplicate samples collected from LOW in May, 1992.

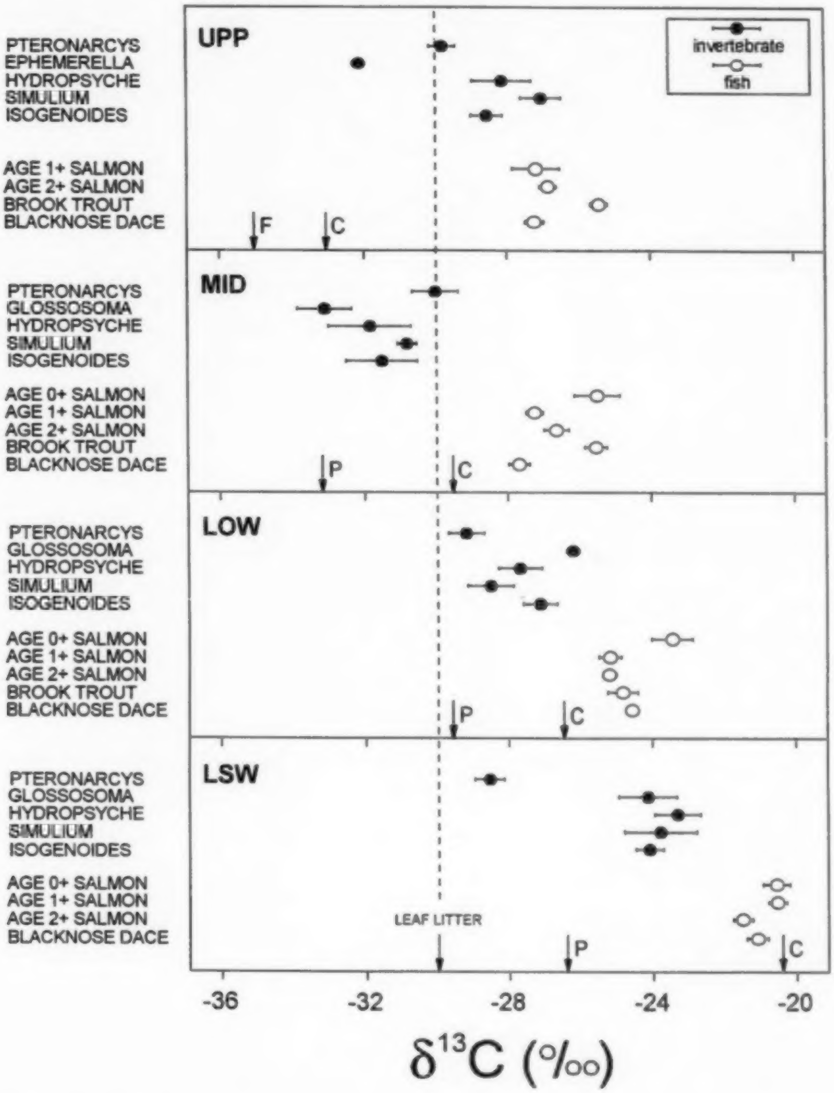
Isotopic mixing model

Because allochthonous carbon can contribute to stream food webs in more than one way (e.g., it may be consumed directly by stream herbivores, in which case it can be considered allochthonous production, or it may be respired to CO_2 and form part of autochthonous production before being utilized by herbivores), we have defined allochthonous carbon in this study as allochthonous production and report the relative importance of this carbon source (e.g., terrestrial leaf litter) to fish at each site using the following equation (modified from Junger and Planas 1994):

$$\%\ \text{allochthonous} = \left(\frac{\delta^{13}C_{\text{fish}} - \delta^{13}C_{\text{autochthonous}} - fx}{\delta^{13}C_{\text{allochthonous}} - \delta^{13}C_{\text{autochthonous}}}\right) \times 100$$

where f is the average trophic enrichment of ^{13}C between an animal

Fig. 2. Stable carbon isotope ratios ($\delta^{13}C$) of invertebrates and fish at four study sites along a stream continuum in the Miramichi River system during the 1992–1993 sampling season (mean ± SE). Arrows refer to site-specific aquatic plant $\delta^{13}C$ values. The vertical broken line represents the average terrestrial leaf litter $\delta^{13}C$ value. C, *Cladophora*; F, *Fontinalis*; P, *Potamogeton*.



and its food (≈1‰; DeNiro and Epstein 1978), and x is the trophic position of the animal (to be determined for each fish species and age-class of salmon using the $\delta^{15}N$ data obtained in this study). $\delta^{13}C_{\text{allochthonous}}$ is the average stable carbon isotope ratio of the sampled leaf litter, which was considered constant at all sites. $\delta^{13}C_{\text{autochthonous}}$ is the site-specific average stable carbon isotope ratio of the algae, *Cladophora*, which was presumed to be representative of the algal carbon signature at each site. This two-source mixing model assumes that leaf litter and algae are the two most important food sources in Catamaran Brook. This hypothesis is based on the general acceptance that mosses and macrophytes are not often used as food by stream consumers (Hynes 1970).

Results

$\delta^{13}C$ of primary food sources

Decomposing leaves from eight of the most common riparian tree species in the Catamaran Brook basin had an average $\delta^{13}C$ of −30.0 ± 0.3‰ (mean ± SE), with conifer needles being slightly more depleted than deciduous leaves (Table 2). *Cladophora* had $\delta^{13}C$ values that varied across the four study sites (range −33.0‰ to −20.3‰) and were strongly correlated with those of DIC (Pearson's $r = 0.95$, $p < 0.0001$), showing a significant trend towards ^{13}C-enrichment in sites located

Table 3. Stable carbon isotope ratios ($\delta^{13}C$) of aquatic plants and dissolved inorganic carbon (DIC) at four sites in the Miramichi River system collected during the 1992–1993 sampling period.

	UPP	MID	LOW	LSW
Dissolved inorganic carbon (DIC)	−13.3 (0.3)	−11.7 (0.1)	−10.5 (0.2)	−8.1 (0.1)
Algae (*Cladophora* sp.)	−33.0 (0.2)	−29.5 (0.2)	−26.6 (0.3)	−20.3 (0.7)
Macrophyte (*Potamogeton* sp.)	np	−32.7 (0.3)	−29.5 (0.3)	−26.7 (0.4)
Moss (*Fontinalis* sp.)	−35.6 (0.3)	np	np	np

Note: Values are means (with SE given in parentheses; $n = 3$). Abbreviations are as in Figure 1.

Table 4. Seasonality of stable carbon isotope ratios ($\delta^{13}C$) of fish collected from four sites in the Miramichi River system collected over the 1992–1993 sampling period.

		$\delta^{13}C$ (‰)				
Site	Date	Age 0+ salmon	Age 1+ salmon	Age 2+ salmon	Brook trout	Blacknose dace
UPP	May	np	−28.1 (—) 1	−26.8 (0.4) 4	−25.2 (0.5) 4	−27.6 (—) 2
	July	np	−27.8 (0.4) 3	−27.0 (0.4) 4	−25.7 (0.3) 5	−26.9 (—) 2
	Aug.	np	−25.8 (—) 2	−26.7 (0.3) 4	−25.2 (0.1) 3	−27.2 (—) 2
MID	May	np	−27.0 (—) 2	−27.9 (0.8) 4	−25.5 (—) 2	−27.5 (—) 2
	July	−21.8 (0.8) 6	−27.6 (0.4) 7	−26.4 (0.4) 9	−26.6 (0.5) 4	−26.8 (—) 2
	Aug.	−27.0 (0.3) 12	−26.9 (0.2) 5	−26.1 (0.8) 5	−25.1 (0.4) 10	−28.0 (—) 8
	Oct.	−27.8 (−) 2	np	−26.8 (—) 2	np np	
LOW	May	np	−26.5 (—) 2	−25.9 (0.7) 4	−24.6 (0.6) 4	−24.9 (—) 2
	July	−20.0 (0.2) 6	−25.5 (0.4) 6	−25.4 (0.3) 6	−24.4 (0.8) 5	−24.4 (0.4) 4
	Aug.	−24.6 (0.4) 12	−23.9 (0.4) 6	−24.9 (0.2) 8	−25.4 (1.0) 7	−24.1 (0.5) 6
	Oct.	−26.5 (−) 2	np	−24.7 (—) 2	np	np
LSW	May	np	np	−20.9 (—) 2	np	np
	July	−20.3 (0.5) 6	−20.7 (0.3) 3	−21.5 (0.2) 9	np	−20.5 (0.1) 3
	Aug.	−21.0 (0.5) 6	−20.4 (0.2) 3	−21.7 (0.4) 7	np	−21.7 (0.3) 3
	Oct.	−21.4 (−) 2	np	−21.7 (—) 2	np	np

Note: Values are means (with SE given in parentheses) and sample sizes. np, not present at the site. Abbreviations are as in Figure 1.

downstream (Table 3). Leaf litter was isotopically distinct from *Cladophora* at all sites (Dunnett's test, $p < 0.05$) except MID ($p = 0.44$), where $\delta^{13}C$ values differed by only ≈0.5‰. *Potamogeton* was always more depleted than *Cladophora* and followed a similar downstream enrichment pattern (Table 3). *Fontinalis* was found only at UPP and was also more depleted than *Cladophora* (Table 3).

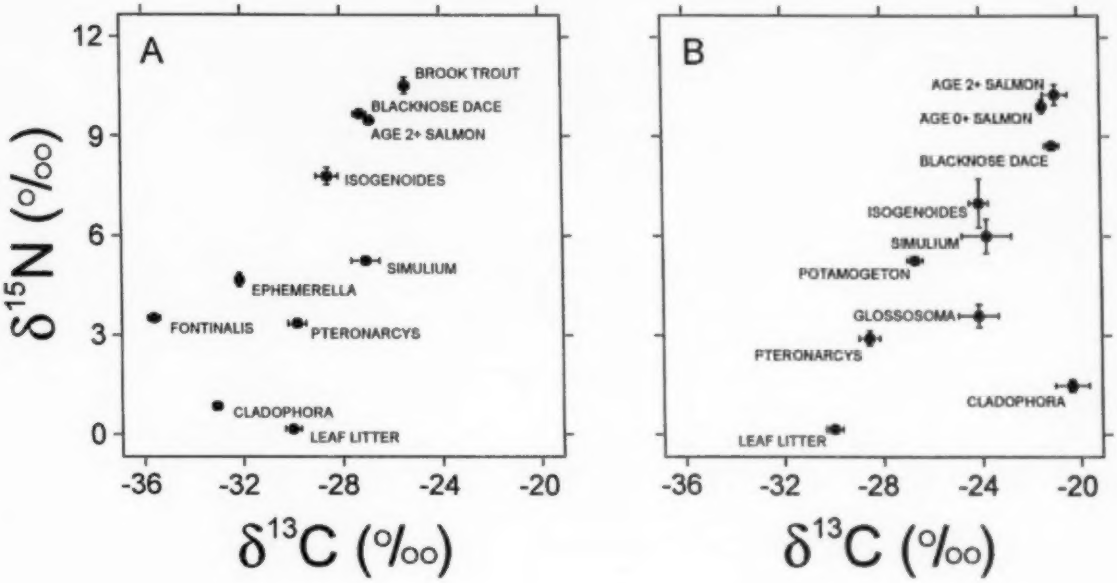
$\delta^{13}C$ of invertebrates and fish

In general, invertebrate $\delta^{13}C$ values in the headwaters of Catamaran Brook (UPP) were more similar to the $\delta^{13}C$ values of terrestrial leaves than to those of aquatic plants (Fig. 2). *Pteronarcys* had a $\delta^{13}C$ value (−29.8‰) close to that of its leaf litter diet, while *Hydropsyche* and *Simulium* were slightly more enriched at −28.1‰ and −27.0‰, respectively. *Ephemerella* was the only taxon to show a distinct algal carbon label at −32.1‰, while *Isogenoides*, possessed an intermediate $\delta^{13}C$ value (−28.6‰), consistent with its carnivorous diet on other invertebrates. Fish $\delta^{13}C$ values (range −28.1‰ to −25.2‰) were similar to, though slightly more enriched than, those of the invertebrate community (Fig. 2). Variability in fish $\delta^{13}C$ was low over the sampling season (Table 4), and brook trout were significantly more enriched than other fishes at this site (Tukey's HSD, $p < 0.01$).

Invertebrates in the middle reach of Catamaran Brook appeared to be less dependent on terrestrial carbon than those collected in the headwaters. At MID, the sampled invertebrate taxa were generally more ^{13}C-depleted (range −33.1‰ to −30.0‰) than leaf litter inputs (Fig. 2). Fish were ≈3–5‰ more enriched than the sampled benthos (Fig. 2). This was a surprising result because animals are typically only ≈1‰ more enriched than their food (DeNiro and Epstein 1978). Seasonality in fish $\delta^{13}C$ was generally low (Table 4), but recently emerged age 0+ salmon (July) were ≈6‰ more enriched than those sampled later in the season. Once again, brook trout were significantly more enriched (−25.5‰) than other fishes (Tukey's HSD, $p < 0.05$) except age 0+ salmon ($p = 0.96$).

Invertebrates collected from the lower reach of Catamaran Brook (LOW) were more enriched than those at MID (Fig. 2). These taxa had $\delta^{13}C$ values (range −29.2‰ to −26.2‰) located between those of terrestrial and aquatic plants, which suggested utilization of both food resources. Fish were also more ^{13}C-enriched (range −25.2‰ to −24.5‰) than those sampled upstream (Fig. 2). Recently emerged age 0+ salmon

Fig. 3. Carbon ($\delta^{13}C$) and nitrogen isotope ($\delta^{15}N$) profiles of plants, invertebrates, and fish collected from (A) the headwaters of Catamaran Brook (UPP) and (B) the Little Southwest Miramichi River (LSW) during the 1992–1993 sampling season. Values are means ± SE.



(July) had much higher $\delta^{13}C$ values than did other fishes (Tukey's HSD, $p < 0.05$) but showed progressive ^{13}C-depletion over the sampling season (Table 4).

Invertebrates and fish from the Little Southwest Miramichi River (LSW) site had the most enriched $\delta^{13}C$ values in this study (Fig. 2). Aside from the distinctly terrestrial label of *Pteronarcys* (–28.5‰), other taxa were less negative (range –24.1‰ to –23.8‰), suggesting greater reliance on aquatic primary production at this site. Fish $\delta^{13}C$ values were also quite high at ≈–21‰ (Fig. 2), and showed a strong dependence on aquatic carbon sources as well. Seasonal differences in fish $\delta^{13}C$ values were not strong, and age 0+ salmon $\delta^{13}C$ values decreased only slightly from July to October (Table 4).

$\delta^{15}N$ profiles

Nitrogen isotopic compositions of plants and animals collected at UPP and LSW are presented in Fig. 3. Leaf litter had the lowest $\delta^{15}N$ value at 0.2‰, while *Cladophora* was slightly higher at 1.5‰. *Potamogeton* and *Fontinalis* were ≈2–3‰ more enriched than *Cladophora*. Animal $\delta^{15}N$ generally increased at successive trophic levels. Primary consumers, such as *Pteronarcys*, *Ephemerella*, and *Glossosoma*, had $\delta^{15}N$ values that ranged from 2.9 to 4.5‰, while *Simulium* was slightly more enriched at 5.4–6.0‰. The predatory stonefly, *Isogenoides*, possessed the highest invertebrate $\delta^{15}N$ values in this study (range 7.0–8.0‰). Fishes were even more enriched than the invertebrate community (range 8.7–10.5‰), and on average, brook trout had higher $\delta^{15}N$ values than either salmon or dace. Because $\delta^{15}N$ values enrich ≈+3‰ per trophic level (Minagawa and Wada 1984), the trophic position of each fish species (x) could be determined and subsequently used in the isotopic mixing model equation to quantify the relative importance of allochthonous carbon in the diets of fishes at each site.

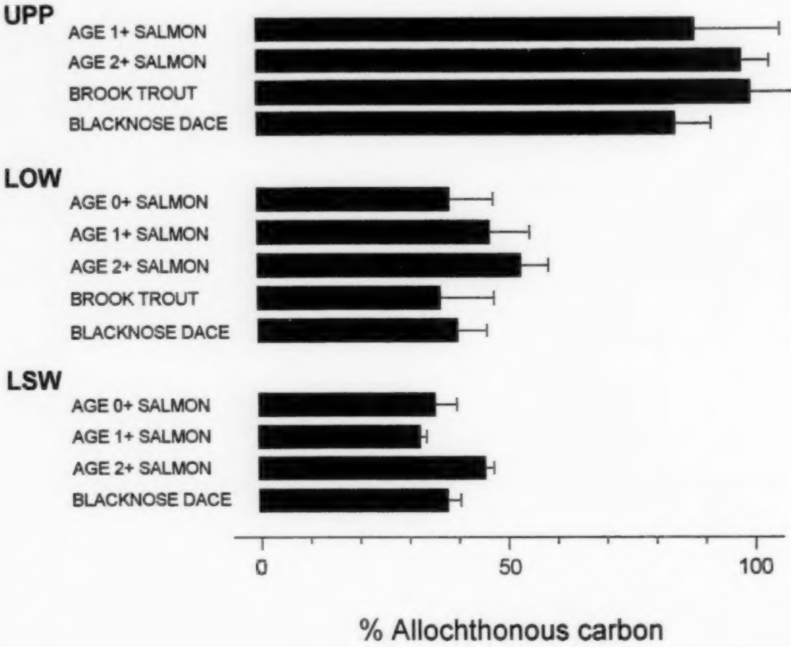
Percent allochthonous carbon

The relative contribution of terrestrial leaf litter inputs (% allochthonous) in the diets of fishes was determined using a two-source isotopic mixing model equation. This model was applied at UPP, LOW, and LSW, where leaf litter and *Cladophora* were shown to be isotopically distinct. Similarity between the $\delta^{13}C$ values of leaf litter and *Cladophora* precluded our attempts to run the model at MID. However, according to the model, fishes at UPP obtained most of their carbon originally from terrestrial sources (Fig. 4). For example, salmon were dependent on terrestrial inputs for 88–98% of their carbon base, while blacknose dace were slightly less dependent at 85%. Brook trout obtained ≈100% of their carbon base from terrestrial sources. At LOW, fishes were less dependent on allochthonous carbon than those in the headwaters, while at LSW fishes derived even less carbon from these inputs (Fig. 4).

Discussion

Our investigations of food webs in Catamaran Brook and the Little Southwest Miramichi River suggest that SIA is a useful tool for measuring the relative contributions of allochthonous and autochthonous carbon in streams. In our study, decomposing leaves were isotopically distinct from algae (*Cladophora*) at all sites except MID. Leaf litter was slightly more depleted than expected for terrestrial C_3 plants, but this likely resulted from the loss of ^{13}C-enriched components during

Fig. 4. Estimates of the relative importance of allochthonous carbon inputs to juvenile Atlantic salmon, brook trout, and blacknose dace at three sites in the Miramichi River system during the 1992–1993 sampling period. Percent allochthonous values were obtained using a two-source isotopic mixing model equation, with terrestrial leaf litter and site-specific *Cladophora* $\delta^{13}C$ values as the two end members. Values are means ± SE.



microbial decomposition (Benner et al. 1987; Rosenfeld and Roff 1992). Differences in *Cladophora* $\delta^{13}C$ among the four sites appeared to reflect a spatial gradient in DIC $\delta^{13}C$, similar to that seen by Kline et al. (1990) and Junger and Planas (1994). The pattern of enrichment in the DIC pool may have resulted from the mixture of ^{13}C-depleted groundwater sources at headwater sites (Oana and Deevey 1960; Rounick and James 1984), with ^{13}C-enriched atmospheric CO_2 (Keeling et al. 1979) along the stream continuum.

Allochthonous carbon appears to be the most important energy source fuelling the benthic fauna in the headwaters of Catamaran Brook. Invertebrates at UPP, with the exception of *Ephemerella*, had $\delta^{13}C$ values that were more similar to those of leaf litter, than to those of either *Cladophora* or *Fontinalis*. Fishes at UPP also had $\delta^{13}C$ values indicative of a greater dependence on leaf litter, and results from the mixing model showed that, on average, >85% of fish carbon was terrestrial in origin. Higher $\delta^{13}C$ values in brook trout may have reflected their larger size (FL ≈ 16 cm) and higher trophic position relative to other fishes at this site (Rau et al. 1983).

More extensive sampling at MID is required before we can determine the primary sources of organic carbon driving this food web. Neither leaf litter nor *Cladophora* was depleted enough to account for the low invertebrate $\delta^{13}C$ values measured at this site. Although *Potamogeton* $\delta^{13}C$ was negative enough to account for these values, it was unlikely to be an important energy source because of its relatively low abundance here or at any of the other study sites. The $\delta^{13}C$ of *Glossosoma*, a caddisfly known to graze specifically on diatoms and other fine materials associated with the upper surfaces of rocks (Tindall and Kovalak 1979), suggested assimilation of a more depleted autochthonous carbon source than that represented by *Cladophora*. Unfortunately, biofilm $\delta^{13}C$ was not measured in this study because of previously reported contamination by terrestrially derived detritus (Winterbourn et al. 1984; Rounick and Hicks 1985; Junger and Planas 1994). Although autochthonous carbon appears to be driving the benthic food web at MID, it remains unclear whether or not invertebrate $\delta^{13}C$ values reflect a more ^{13}C-depleted algal carbon source than *Cladophora*, or a detrital link between *Potamogeton* and the benthic food web.

Differences between fish and benthic invertebrate $\delta^{13}C$ values at MID require further investigation as well. Fish were generally too ^{13}C enriched to have derived a significant amount of their carbon base from the sampled invertebrate taxa. It may be that fish were feeding either on invertebrates drifting down from less-depleted upstream areas or on terrestrial insects with distinctly allochthonous carbon labels. Examination of gut contents, however, revealed only small proportions of terrestrial insects in the diets of most fishes at this site (Doucett 1994).

The faunal $\delta^{13}C$ data at LOW and LSW confirm the increasing importance of autochthonous primary production at downstream sites, as would be predicted by the river continuum concept (Vannote et al. 1980). Invertebrate $\delta^{13}C$ values in the lower reach of Catamaran Brook fell between those of leaf litter and *Cladophora*, suggesting a dependence on both resources. Fishes were ^{13}C enriched relative to those located upstream, and exhibited a 39–53% dependence on terrestrial carbon according to the mixing-model equation. High invertebrate δ^{13}C values at LSW suggested that biomass carbon must have been derived mostly from in-situ sources. Autochthonous carbon predominance was also apparent in the fish community at this site, which derived 33–46% of their carbon from terrestrial sources.

δ^{15}N profiles clearly identify food web structure in the headwaters of Catamaran Brook and in the Little Southwest Miramichi River. Despite obvious differences in carbon pathways, nitrogen isotope distributions were almost identical at UPP and LSW, indicating similarity in the δ^{15}N compositions of the primary energy sources and in the patterns of isotopic fractionation within each food chain. Fishes had δ^{15}N values that were $\approx$8–10‰ above the primary food sources, suggesting 2.5–3.5 trophic levels in these streams. These findings were consistent with estimates of stream trophic structure from other studies using δ^{15}N values (Estep and Vigg 1985; Fry 1991).

Seasonal changes in the δ^{13}C of age 0+ salmon emphasize the value of SIA for investigating temporal changes in the energetic pathways of fish communities. Maternally derived marine carbon was likely responsible for higher δ^{13}C values in postemergent age 0+ salmon. Because Atlantic salmon migrate to the open ocean to grow and mature, adults returning to spawn in Catamaran Brook possess δ^{13}C values ($\approx$–18‰; Doucett 1994) that reflect marine primary production ($\approx$–21‰; Peterson and Fry 1987). Subsequent dilution of stored marine carbon in the tissues of recently emerged age 0+ salmon following feeding in freshwater likely resulted in more ^{13}C-depleted values later in the growing season.

In contrast, lack of seasonality in the δ^{13}C values of age 1+ and age 2+ salmon suggest that isotope ratios in older parr reflect longer term diet. Turnover in animal δ^{13}C is related to growth rate (Fry and Arnold 1982; Tieszen et al. 1983), and older fish take longer to reflect changes in dietary δ^{13}C than smaller fish (Hesslein et al. 1993). Therefore, unlike the carbon pool of age 0+ salmon, which is either turned over or diluted during the summer months, the δ^{13}C values of older parr represent food sources assimilated over two or more seasons. Obtaining estimates of carbon turnover times for different age-classes of Atlantic salmon parr will be a crucial step in understanding potential changes in fish δ^{13}C values that may follow deforestation in the Catamaran Brook basin.

SIA appears to have great potential as an effective fisheries management tool because it provides a means by which the energy basis for secondary production can be determined. In this study, the relative importance of allochthonous carbon in stream food webs leading to wild juvenile Atlantic salmon was quantified using a combination of stable carbon and nitrogen isotope ratios. Estimates from an isotopic mixing model equation showed that fishes obtained most of their carbon from allochthonous sources in the headwaters, but in downstream sites, autochthonous inputs played an equally important role. These results are consistent with the river continuum concept (Vannote et al. 1980). Small forested streams, such as Catamaran Brook, are closely linked to their surrounding catchments, and inputs from the terrestrial environment are regarded as principal energy sources fuelling benthic communities in headwater sites (Hynes 1975). Moving downstream into the Little Southwest Miramichi River, open canopies allow increased solar radiation to stimulate higher autochthonous production. A similar study on a small New Zealand catchment (Rounick et al. 1982; Rounick and Winterbourn 1986) showed that SIA has the potential to resolve changes in the nature of the food base following forest removal. In this study, *Deleatidium*, a browsing mayfly, came to depend more on autochthonous carbon sources over a 9-year period after cutting. As the Catamaran Brook basin is scheduled for logging in 1996–1999 (Cunjak et al. 1990), continued sampling efforts should show the value of the SIA technique for investigating trophic responses of fish communities to environmental change.

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Effects of sperm irradiation and heat shock on induction of gynogenesis in muskellunge (*Esox masquinongy*)[1]

Feng Lin and Konrad Dabrowski

Abstract: We report here the first induction of gynogenesis in esocid fish by UV irradiation and heat shock. Optimal dosage of sperm UV irradiation was in the range of 1440–2160 J/m^2. Eggs fertilized with irradiated sperm at such dosages and not heat shocked yielded 100% abnormal larvae with the fertilization rate at 48.3 ± 8.4% (mean ± SD). There were no significant differences in survival to the eyed stage under the heat shock conditions tested (5–30 min post-fertilization for 5–30 min at 26–30°C). Gynogens were induced in most of the groups with hatching rates at 0.2–3.3%. Optimal conditions for gynogenetic induction of muskellunge (*Esox masquinongy*) were the application of 30°C for 8–10 min beginning 20 min post-fertilization, with a yield of gynogens at 5.8–6.0% (hatched normal larvae/viable eyed-stage embryos). Ploidy was identified by chromosome preparation, silver staining for nucleolar organizer regions, and flow cytometry measurement of the nuclear DNA content using eyed-stage embryos and larvae. Haploids hatched but died within 1 week.

Résumé : Dans cet article, nous faisons état de la première gynogénèse induite chez les Éspcodés par rayonnement UV et choc thermique. La dose optimale d'irradiation du sperme était de l'ordre de 1440 à 2160 J/m^2. Les oeufs fécondés avec le sperme irradié à ces doses et qui n'ont pas reçu de choc thermique donnaient 100 % de larves anormales à un taux de fécondation de 48,3 ± 8,4% (moyenne ± écart standard). Nous n'avons observé aucune différence significative au niveau de la survie jusqu'au stade embryonné dans les conditions de choc thermique utilisées (5–30 min après la fécondation, pendant 5–30 min, à 26–30°C). Des sujets gynogénotes ont été obtenus dans la plupart des groupes à un taux d'éclosion de 0,2–3,3 %. Chez le maskinogé (*Esox masquinongy*), les conditions optimales pour l'induction de la gynogénèse sont l'application de la chaleur (30°C pendant 8–10 min, traitement débutant 20 min après la fécondation); dans ces conditions, le rendement en sujets gynogénotes est de 5,8–6,0 % (larves normales écloses/embryons viables jusqu'au stade oeillé). Le degré de ploïdie a été déterminé par la préparation des chromosomes, la coloration argentique des régions des organisateurs nucléolaires et par la mesure par cytométrie de flux du contenu en ADN nucléolaires et par la mesure par cytométrie de flux du contenu en ADN nucléaire des embryons oeillés ou des larves. Les sujets haploïdes naissaient, mais mour aient en 1 semaine.
[Traduit par la Rédaction]

Introduction

Gynogenesis in fish has been studied intensively in the past few decades for its potential value in experimental genetics and aquaculture (see reviews by Thorgaard 1983; Ihssen et al. 1990). Gynogenesis can be achieved by retention of the second polar body or suppression of the first mitotic division of eggs inseminated with irradiated spermatozoa. Gynogenetic fish have been successfully produced in many species, such as rainbow trout (*Oncorhynchus mykiss*) (Chourrout 1982), common carp (*Cyprinus carpio*) (Komen et al. 1988), tilapia (*Oreochromis mossambicus*) (Varadaraj 1990), yellow perch (*Perca flavescens*) (Malison et al. 1993), and loach (*Misgurnus anguillicaudatus*) (Arai et al. 1993). Spermatozoa from related fish species can also be used to initiate embryonic development (Chourrout 1986). However, low survival of eggs activated and treated to produce gynogens coupled with inbreeding deficiency limit the direct application of this technique (Dunham 1990). A combination of gynogenesis and sex reversal can be used to overcome these problems. This procedure has also been used for producing all-female populations of grass carp (*Ctenopharyngodon idella*) (Shelton 1986).

Muskellunge (*Esox masquinongy*) is one of the most popular sport fishes in the north-central United States and central Canada. Annual stocking of this species is required to meet the demands of anglers because of its failure to reproduce naturally in many reservoirs (Dombeck et al. 1984). Like many economically important cool-water species, muskellunge exhibits sexually related dimorphic growth (Scott and Crossman 1973), where femal es reach total length of 760 mm in 3 years, whereas males require 4 years in Clear Fork Reservoir, Ohio (Day and Stevenson 1989). Males have a higher mortality than females at age 4 and older. Thus, stocking all female muskellunge potentially increases stocking efficiency thereby benefiting the sport fishery. Unfortunately, little knowledge of reproductive physiology and genetics of

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this species is available. McGregor (1970) described karyotypes of muskellunge with an air-drying technique using kidney cells from juvenile fish. Galat (1973) reported the gross normal embryonic development from fertilization until hatching by analyzing preserved specimens, while Leslie and Gorrie (1984) gave a general description of the early life history of muskellunge.

The aim of our study was to investigate methods for producing all-female muskellunge by using gynogenesis. We evaluated effects of temperature and storage times on the viability of muskellunge eggs to facilitate fertilization in controlled, laboratory conditions. We report here the induction of gynogenetic muskellunge by UV irradiation of sperm and heat shock of embryos.

Materials and methods

Gravid muskellunge females and running ripe males were captured by trap nets from the Clear Fork Reservoir, Ohio, in 1993 and 1994. The captured fish were maintained in a floating pen for a few hours before being stripped of gametes. Gametes were collected from unanesthetized fish, and semen was kept on ice before use. Eggs were kept in a foam box where, as a result of a storage experiment, temperature was maintained at approximately 12°C. Sperm motility was checked before experimentation (Lin and Dabrowski 1996), and only semen with >90% initial motility was used for irradiation or fertilization. Semen from three to five males and eggs from two or three females were pooled before fertilization. Eggs were fertilized with fresh semen to serve as a control for gamete quality in each experiment. Survival to the eyed stage was obtained by counting and removing white (dead) eggs at 7–9 days post-fertilization.

Egg handling and storage

Previous experience revealed that storage of muskellunge eggs directly on ice during transportation reduced their quality. Hence, an experiment quantifying temperature effect and duration of storage on egg quality was conducted. Eggs from three individual females (about 600 eggs from each) were fertilized with freshly collected sperm at Clear Fork Reservoir immediately after egg collection. Eggs pooled from three females were kept in sealed plastic bags filled with oxygen immediately after collection at Clear Fork Reservoir. Three bags (each with about 100 mL of eggs) were then dispersed among three shelves of a foam box with ice in the bottom shelf, thus creating three levels of temperature. Three subsamples of eggs were removed from the bags and fertilized after 2, 5, and 8 h of storage, and the temperatures of the eggs were recorded. The temperatures of eggs on different shelves were 13.6–16.7, 10.1–13.1, and 6.0–7.1°C during the experiment. Replicated samples of eggs were incubated separately in PVC jars with a screen bottom, which were set up in California-type hatching trays (Flex-a-lite Consolidated, Tacoma, Wash.). Water temperature was 12–13°C during incubation. Survival of embryos to the eyed stage, approximately 6–7 days, was used as a criterion to evaluate egg quality.

UV irradiation for sperm inactivation

Semen (0.5 mL) was diluted with 4.5 mL of 0.75% NaCl and 0.6% KCl (Varadaraj 1990). A portion (0.5 mL) of diluted semen was placed on ice to serve as a control for the effect of dilution and storage on sperm fertility. Approximately 300 eggs were fertilized with 0.25 mL of diluted semen immediately (control 1) and 20 min after dilution (control 2). The remaining diluted semen was poured into a watch glass and UV irradiated with a short wave (254 nm) mineralight lamp (Model UVG-11, UVP, Inc., San Gabriel, Calif.) at 6 W/m^2. The intensity of UV irradiation was measured using an UVX radiometer (UVP, Inc., San Gabriel, Calif.) with a sensor placed at the same level as the sperm suspension. Sperm suspension was kept on ice and continuously stirred with a magnetic stirrer during the irradiation. Subsamples (0.25 mL) of the sperm suspension were removed and used to fertilize batches of 300 eggs at 2, 4, 6, 8, 12, 16, 20, and 30 min after dilution and UV irradiation. After 3–5 min of water hardening, each batch of eggs was further divided into three groups and incubated in California-type hatching trays with recirculating water at 12°C. The fertilized eggs were kept in the dark because damaged sperm DNA could be partially photorepaired by exposure to visible light (Thorgaard 1983). Survival to the eyed stage and hatching were recorded with abnormal larvae considered as mortality.

Table 1. Schedule for the effects of temperature and duration of heat shock starting 20 min post-fertilization at 12°C.

Temperature (°C)	Duration of heat shock (min)				
26	10	15	20	25	30
28	6	8	10	15	20
30	2	4	6	8	10

Heat shock to inhibit the second meiosis

Eggs were fertilized with irradiated spermatozoa (2000 J/m^2) as described above. After water hardening, eggs were divided into PVC jars with a screen bottom. The first experiment was designed to estimate effects of initial time of heat shock on polar body retention. The jars were transferred to temperature regulated water baths of 26 and 28°C at 5, 10, 15, 20, 25, and 30 min post-fertilization for 10 and 20 min, then returned to the hatching trays for incubation. Fluctuation of water temperature was controlled within 0.1°C, and water was circulated to ensure a homogeneous temperature in each bath. A second experiment was designed to determine optimal temperature level and duration of heat shock. Eggs were shocked at three temperatures for various times, starting at 20 min post-fertilization (Table 1). One jar that was not heat shocked served as a negative control. Another batch of eggs was fertilized with diluted semen (4 min on ice) as a control for eggs and sperm quality. Eggs were incubated and survival to the eyed stage and hatching was estimated.

Chromosome preparation and silver staining for nucleolar organizer regions

Slides were prepared by a modified method of Kligeman and Bloom (1977) and Chourrout and Happe (1986). Briefly, eyed-stage embryos were incubated in 0.02% colchicine overnight, then the embryos were dissected from the yolk in physiological saline. Embryos were fixed with ethanol : acetic acid (3:1) for at least 2 h on ice after a hypotonic treatment in 0.8% sodium citrate for 30 min at room temperature. Individual embryos were minced in 50% acetic acid and the cell suspension was dropped onto prewarmed slides. Slides were stained in 10% Giemsa for chromosomes. Slides for nucleolar organizer regions (NORs) were prepared in the same way, or without colchicine and hypotonic treatment, as for chromosome preparation. Silver staining followed the method of Phillips et al. (1986). The number of nucleoli from 200 randomly selected cells was recorded in each of 6 abnormal fish as well as 6 control fish.

Flow cytometry measurement of DNA content

Random samples from several groups, including normal and abnormal embryos and larvae, were examined. Embryos and larvae were dissected from their yolks and individually added to 1 mL incubation solution in a 12 × 75 mm culture tube (Malison et al. 1993). The incubation solution contained 50 mg propidium iodide and 10 mg ribonuclease A in 1 L Hematall (Fisher, catalogue No. CS606-20). Rainbow trout red blood cells were used as an external standard as well as added to the incubation solution as an internal standard. Fresh blood was collected from an individual rainbow trout for all measurements. Stained embryos and larvae were syringed through 21- and 25-g needles after overnight incubation at dark in a 4°C refrigerator. Cell suspensions were filtered through 60-μm nylon filters before being run through an Elite flow cytometer (Coulter Corporation, Hialeah, Fla). Fluorescence, forward scatter, and 90° scatter were measured and data were stored as a listmode file. At least 10 000 events (cells) were recorded for each sample. Electronic gating was applied to exclude the doublets in calculating the distribution of fluorescence. Cell cycle was analyzed with a computer program, Multicycle (Phoenix Software, San Diego, Calif.).

Data analysis

All values were expressed as mean and standard deviation. Statistical significance was assessed by a two-way analysis of variance (ANOVA) using the GLM procedure in SAS^{TM} version 6.07 (SAS Institute Inc., SAS Campus Drive, Cary, N.C.).

Results

Embryo survival in muskellunge eggs fertilized immediately after gamete collection amounted to 58.9 ± 17.9% (*n* = 3 females). We did not include these data in statistical comparisons with experimental treatments because individual rather than pooled samples of eggs were incubated. Eggs stored at intermediate temperatures (10–13°C) had the highest survival rate to the eyed stage (Fig. 1). Muskellunge eggs stored for 8 h at 10–13°C had less than 30% mortality. This temperature range was the intermediate water temperature at the capture sites over the season (8.5–15.0°C). Low temperature (6–7°C) significantly increased egg mortality ($P < 0.01$). High temperature, 13.6–16.7°C, had a less negative impact on egg fertility and survival than low temperature.

In the experiment using UV irradiation to inactivate the sperm, eggs fertilized with fresh undiluted semen that served as egg quality controls had 84.6 ± 2.5% survival to the eyed stage. Survival to the eyed stage was 43.4 ± 2.9% for control 1 (fertilized immediately after dilution; Fig. 2) and 12.9 ± 0.5% for control 2 (fertilized 20 min after dilution). Irradiation at a dose greater than 720 J/m^2 (2 min) resulted in total abnormal embryonic development. Eggs inseminated with sperm irradiated for 6 min had the highest survival to the eyed stage (54.7 ± 4.2%). Abnormal embryos can be identified morphologically starting from 7 to 9 days post-fertilization based on embryo size and shape, malformed eye, shortened body, and curved tail region (Fig. 3A). Most of the abnormal embryos hatched, but larvae were small and deformed (Fig. 3C) and died within a week.

Chromosome preparations verified that abnormal embryos were haploid, *n* = 25, whereas normal embryos had 50 chromosomes. Silver staining for NORs showed that cells of abnormal embryos and larvae had only one (89.9 ± 4.5%) or no NORs (10.1 ± 4.5%), whereas cells from normal embryos and larvae had two (51.3 ± 1.7%), one (41.5 ± 2.7%), or no NORs (7.2 ± 2.1%) (*n* = 6; Fig. 4). The maximum number of nucleoli was one for the haploid cells, and two for the diploid cells. Thus, silver staining for NORs could be a reliable method for ploidy identification.

The fluorescent peak from normal embryos and larvae had a mean channel number of 308 (relative DNA content), whereas the peak from abnormal embryos and larvae had a mean of 160 (Fig. 5). The DNA content of haploid cells was half (0.507) that of diploid cells after calibration with internal standards. This supported NOR data that the abnormal embryos and larvae were haploids. All the normal embryos and larvae had a diploid peak while all the abnormal embryos and larvae had a haploid peak (n = 45). Cell cycle analysis by computer revealed that the haploid cells had a substantial G_2 peak, cells in the interphase II and mitosis of a cell cycle. The cells at each stage of the cell cycle were 82.0, 11.9, and 5.1% for G_1 (interphase I), S (DNA synthesis), and G_2, respectively. The G_2/G_1 ratio, representing relative DNA content of these two cell populations, was 1.979. Electronic gating ensured that this peak did not result from cell aggregation. However, such G_2 peaks were not detected in normal diploid cells. Data used for cell cycle analysis were collected without an internal standard to avoid interference from the standard peak of rainbow trout red blood cells.

In the heat shock experiments, eggs fertilized with diluted but not irradiated semen, which served as an egg quality control had an average survival to eyed stage of 77.7 ± 7.5%. Eggs in all heat shock groups as well as negative control, groups that were activated by UV irradiated sperm and not heat shocked had similar survival to the eyed stage in both experiments (Figs. 6 and 7). There was no significant difference in survival to the eyed stage among all experimental groups. This indicated that heat shock under such conditions did not have a severe impact on egg viability. However, the average survival of all experimental groups, 58.0 ± 11.1% (Fig. 7), was significantly lower than that of controls with non-irradiated semen (77.7%). This suggested that the process of semen irradiation rather than heat shock was the major cause for increased mortality.

All embryos in the negative control group hatched as abnormal larvae. This indicated that the UV irradiation effectively destroyed the genetic material of the sperm but retained the ability of sperm to induce the development of eggs. Such eggs developed abnormally without subsequent heat shock but normally when heat shock was given. Further, the abnormal embryos and larvae were shown to be haploids by flow cytometery, while the normal embryos and larvae were diploids. Therefore, the heat shock must have caused duplication of chromosomes in eggs fertilized with UV irradiated sperm.

Gynogens were induced in most of the heat shock groups with hatching rates of 0.2–3.3%. The yield of gynogens was the ratio of hatched normal larvae to the viable eyed stage embryos. The highest yield of gynogens was 6.0% with heat shock at 30°C for 8 min starting at 20 min post-fertilization (Fig. 8). There was a significant effect of heat shock temperature and time after fertilization on yield of gynogens ($P <$ 0.01) (Figs. 8 and 9).

Discussion

Fertilization rates from eggs pooled from several females should represent the mean for the population being sampled. There is no evidence in the literature that pooling samples of eggs from several females influences their fertilization rate in comparison with the average fertilization rate of eggs from individual females. We conclude that the fertilization rate of fresh gametes and that of gametes stored at the optimal tem-

Fig. 1. Effects of temperature and duration of egg storage on embryo survival. Temperatures were high: 13.6–16.7°C; middle: 10.1–13.1°C; low: 6.0–7.1°C. Eggs were fertilized at 2, 5, and 8 h after collection. Bars with the same letters are not significantly different at $P = 0.05$.

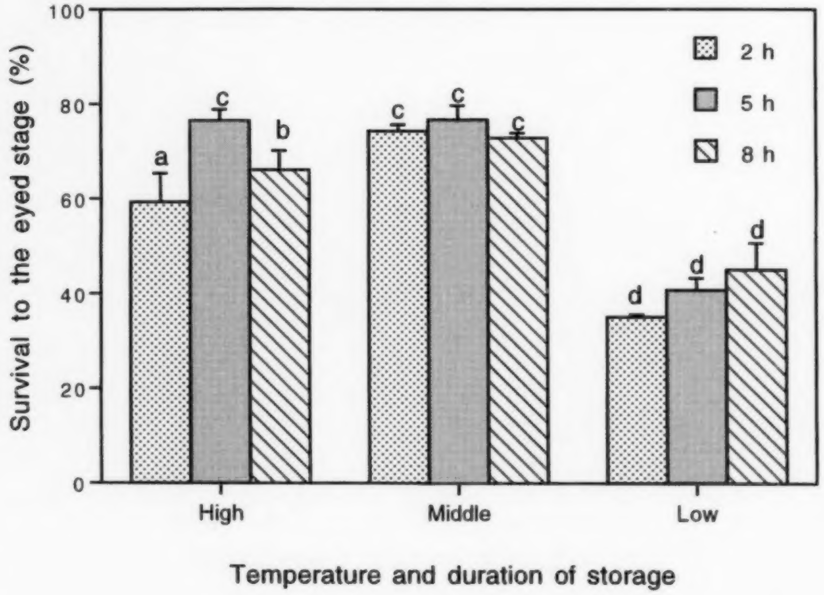
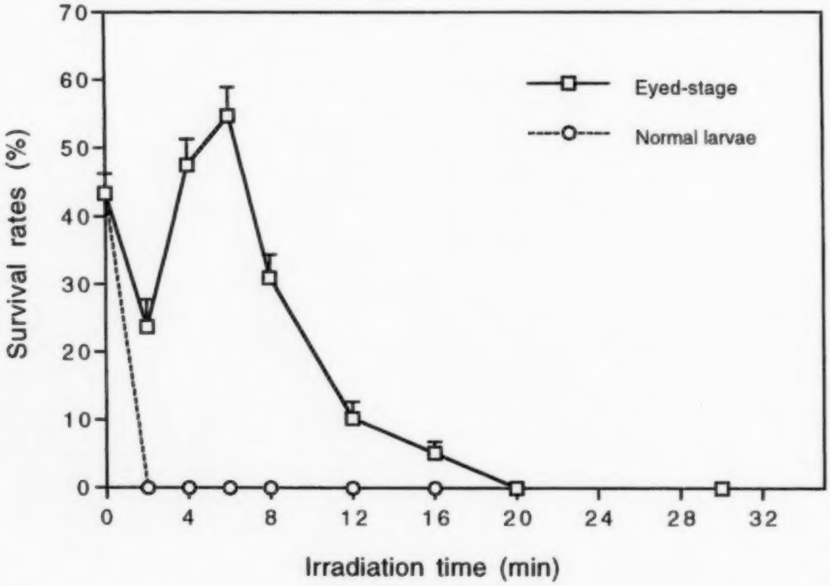


Fig. 2. Effects of dilution and irradiation time on survival of eggs inseminated with irradiated spermatozoa. UV intensity was 6 W/m^2. Larvae with deformed bodies were not counted as normal hatching. Eggs fertilized with semen immediately after dilution without any UV exposure are shown at 0 irradiation time (control 1). Eggs fertilized with semen 20 min after dilution but not irradiated (control 2) had 12.9 ± 0.5% survival (not shown).



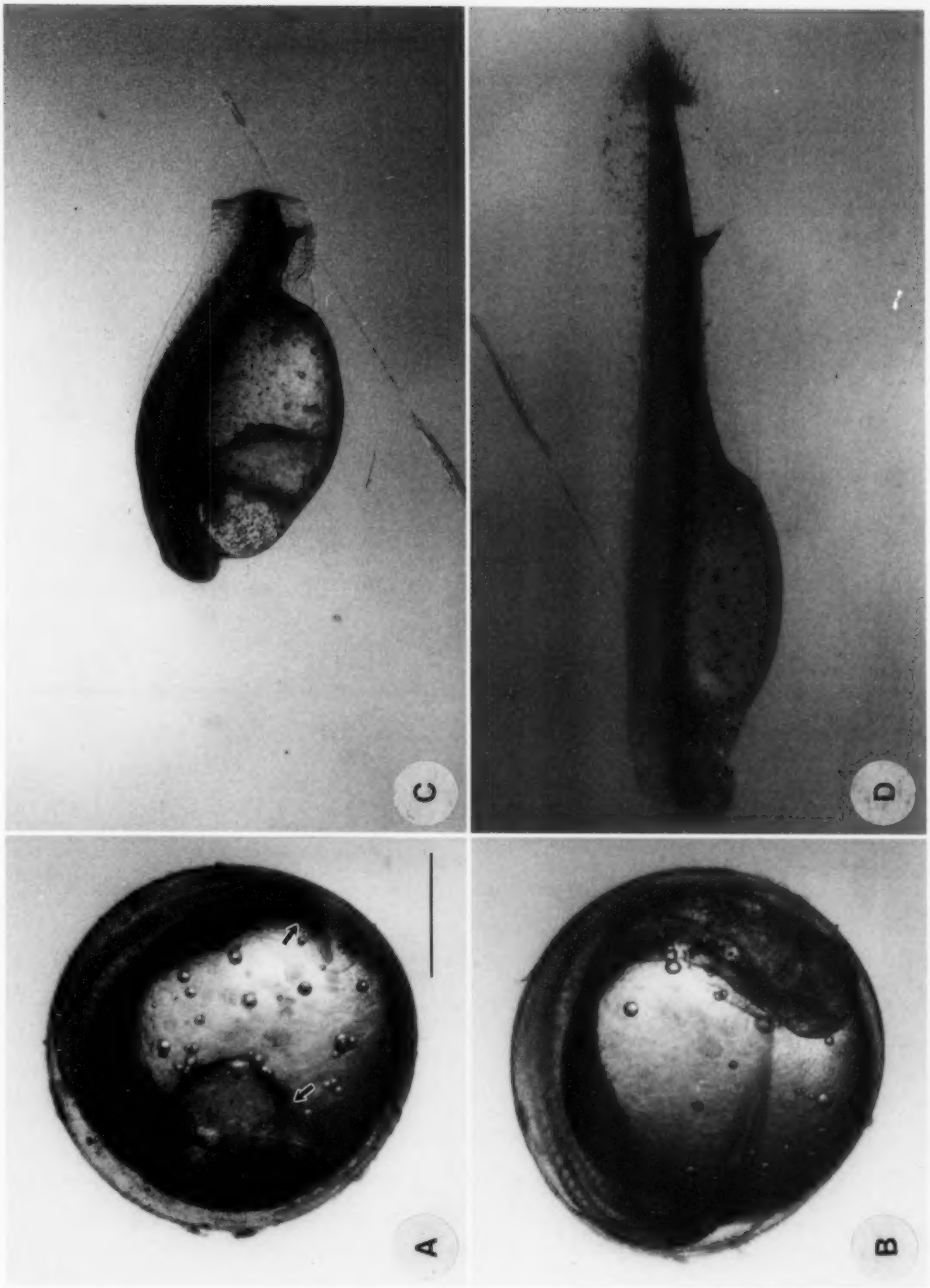
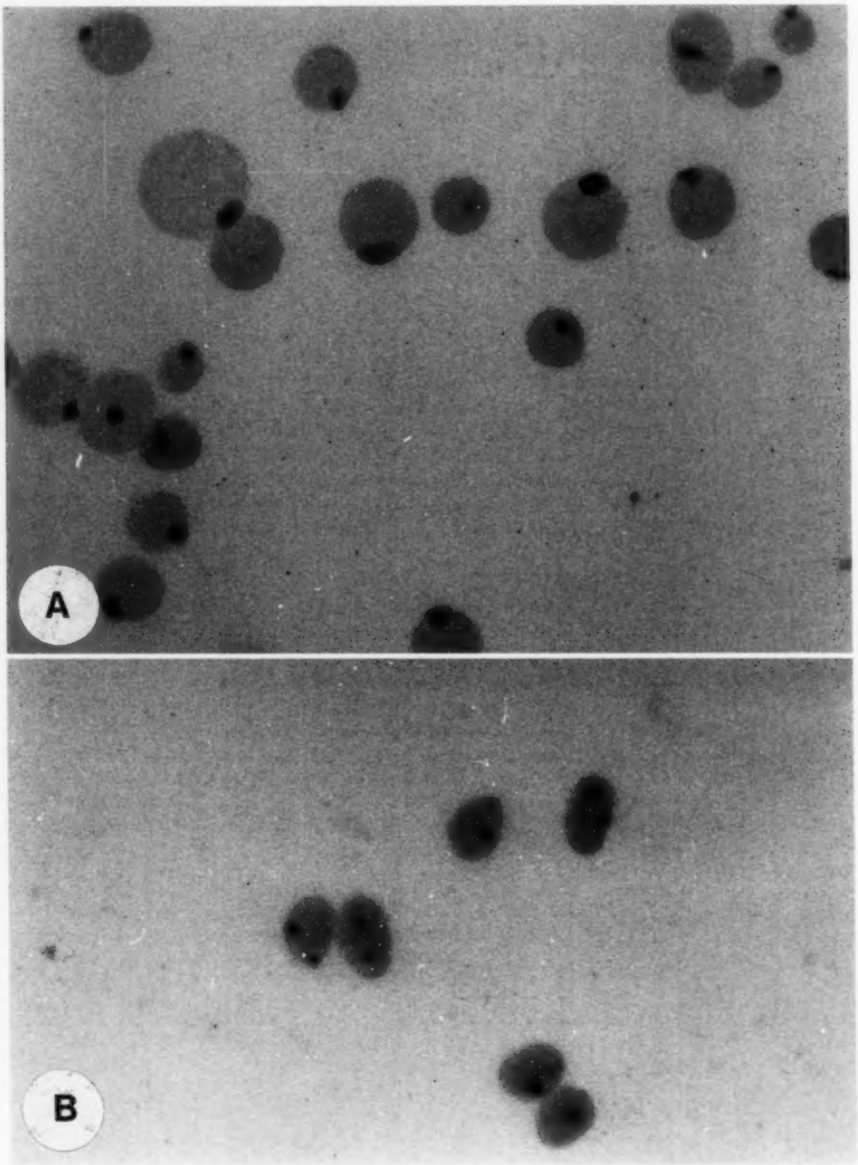


Fig. 3. Morphology of abnormal haploid (A, C) and normal diploid (B, D) muskellunge embryos and larvae. All abnormal larvae died within 1 week. Arrows indicate abnormal cell proliferations. Scale bars are 1 mm in Figs. 3A and 3B and 2 mm in Figs. 3C and 3D.

Fig. 4. Nucleolar organizer regions of diploid and haploid cells prepared from normal and abnormal eyed stage embryos: (A) haploid, with a maximum of one nucleolus; (B) diploid, with a maximum of two nucleoli.



perature in all our experiments was comparable. The optimal temperature of egg storage for muskellunge was 10–13°C from the egg storage experiment. Thus, we chose 12°C as the temperature for the storage of eggs in our other experiments. Sperm of muskellunge stored on ice for 5 h and used for fertilization resulted in 76.6 ± 3.1% (*n* = 3) survival to the eyed stage (Fig. 1), close to the average rate of fertilization obtained from other experiments in our laboratory (78%; Lin et al. 1996). There was no significant difference in survival of embryos between 2 and 8 h of storage (both eggs and sperm) within intermediate- and low-temperature groups. Therefore, we conclude that the impact of duration of sperm storage on ice in this experiment was negligible.

Regarding temporary storage of eggs, Kuchnow and Foster (1976) reported a similar finding to ours in mummichog (*Fundulus heteroclitus*). They found fertility of eggs remained high for 24 h at the optimal temperature range (6–10°C), whereas eggs showed reduced or no fertility when

Fig. 5. Representative fluorescent distribution of cells prepared from diploid and haploid embryos measured by a flow cytometer. Rainbow trout red blood cells were used as an internal standard. The haploid peak of muskellunge had a mean fluorescence intensity at channel No. 160; the diploid peak of muskellunge had a mean fluorescence intensity at channel No. 308; and the internal control of rainbow trout red blood cells had a mean fluorescence intensity at channel No. 642.

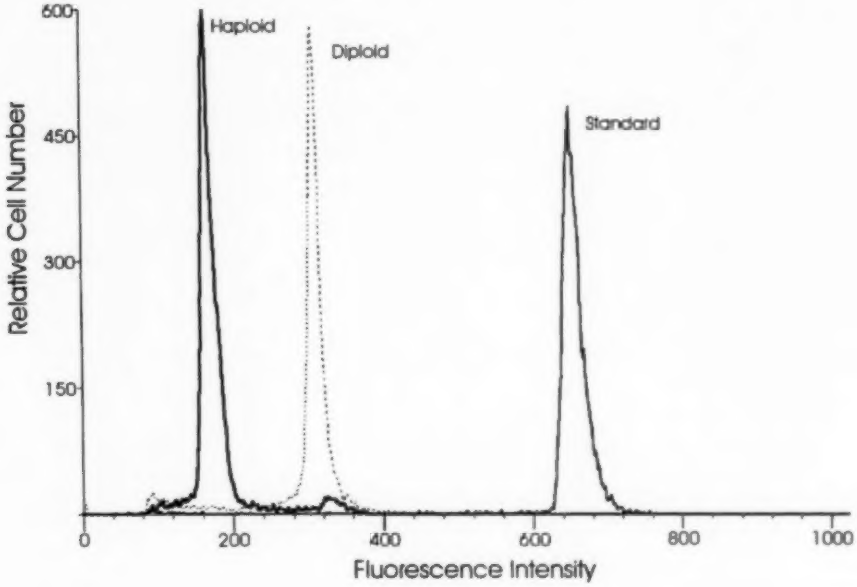


Fig. 6. Effects of initial time of heat shock on the survival to the eyed stage. Eggs fertilized with UV irradiated spermatozoa were heat shocked at 26 and 28°C for 10 and 20 min starting at different post-fertilization times. Control (C) was a negative control with UV irradiated sperm but not heat shock.

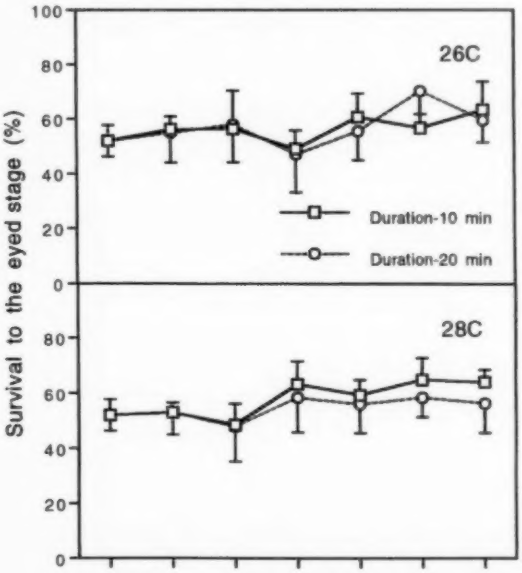
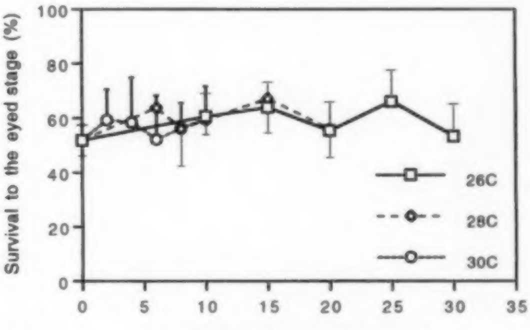


Fig. 7. Effects of temperature and duration of heat shock on the survival to the eyed stage. Eggs that were fertilized with UV irradiated spermatozoa were heat shocked starting at 20 min post-fertilization at 26, 28, and 30°C for various durations according to Table 1. Duration time of zero was the negative control for UV irradiation.



stored at temperatures below 6°C. Low storage temperature was not suitable for sturgeon (*Acipenser guldenstadti*), which showed no fertility of eggs stored at 0°C (Ginsburg 1972; cited in Stoss 1983). However, storage of salmonid eggs at temperatures of 0–4°C has always been superior to storage at a higher temperature. Salmonid eggs of several species have been successfully stored for a few days in ovarian fluid at low temperatures (see review by Stoss 1983). There is yet no

Fig. 8. Effects of temperature and duration of heat shock on yield of gynogens. Eggs that were fertilized with UV irradiated spermatozoa were heat shocked starting at 20 min post-fertilization at 26, 28, and 30°C for various durations according to Table 1. Duration time of zero was the negative control for UV irradiation.

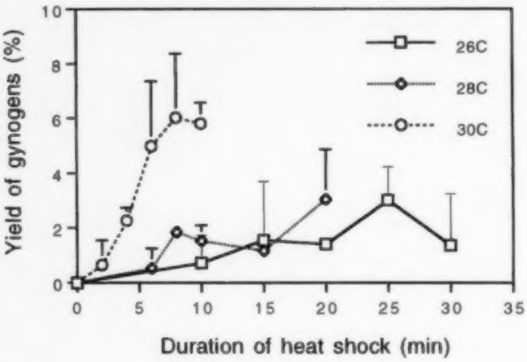
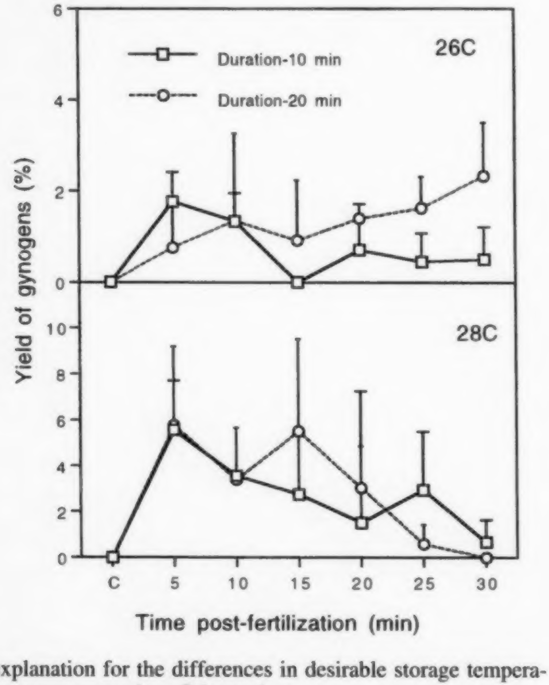


Fig. 9. Effects of initial time of heat shock on yield of gynogens. Eggs fertilized with UV irradiated spermatozoa were heat shocked at 26 and 28°C for 10 and 20 min starting at different post-fertilization times. Control (C) was negative control with UV irradiated sperm but not heat shock. Yield of gynogens is the ratio of normal larvae to the viable eyed stage embryos.



explanation for the differences in desirable storage temperatures among various fish species.

The optimal condition for UV irradiation was considered to be in the range of 1440–2160 J/m^2 (4–6 min) with a fertilization rate at 48.8 ± 8.4%. At this UV exposure, sperm retained their ability to activate egg development while sperm DNA was inactivated completely. At a low dosage of 720 J/m^2 (2 min), genetic material of sperm might not be completely destroyed and interfere with embryonic development, thus causing relatively low survival to the eyed stage. We found that UV irradiation of eggs (for androgenesis) at a marginal dosage resulted in malformations in embryonic development and simultaneous decrease in DNA content in comparison with diploid controls (F. Lin and K. Dabrowski, unpublished data). This phenomenon revealed a typical "Hertwig effect" (Thorgaard 1983), when haploid embryos resulting from total destruction of the genetic material in the sperm by high levels of radiation would survive longer than diploid embryos expressing dominant lethal mutations induced by radiation. Prolonged exposure to UV (>8 min) and stirring during irradiation reduced the insemination ability of sperm (Fig. 2). The decreasing survival of eggs fertilized with semen immediately after dilution (control 1) and 20 min later (control 2) indicated that dilution and keeping the diluted semen on ice had a negative impact on sperm fertility. Thus, duration of irradiation should be kept short to avoid loss of sperm fertility. A high-power UV lamp can be used to improve efficiency of exposure and to shorten the irradiation time. A better diluent for sperm irradiation is also desirable.

We verified that all normal hatched larvae examined, using chromosome preparation, silver staining of NORs, and flow cytometry measurement of DNA content, were diploids while all abnormal larvae were haploids. Thus, haploids and diploids could be identified by their morphology. The chromosome preparation for muskellunge is time-consuming and well-spread metaphase chromosomes can only be obtained during a specific period of embryonic development. A similar situation was reported in rainbow trout (Chourrout and Happe 1986). We provided evidence that silver staining of NORs was a reliable method for ploidy identification in muskellunge with each set of chromosomes containing only one NOR in this species. Silver staining of NORs has been used to identify ploidy levels in salmonids (Phillips et al. 1986), cyprinids (Sola et al. 1986; Carman et al. 1992), and most recently in comparing different species of esocoid (Rab and Crossmon 1994).

Flow cytometry measurement of DNA content is a quick and accurate method for ploidy identification. It has several advantages over other methods because it can also detect aneuploidy and mosaic individuals. We found that a significant percentage of G_2 peak (5.1%) was present in haploid individuals, whereas G_2 peak was undetectable in the diploid individuals. Since electronic gating eliminated the possibility of doublets in cell suspension of haploids, another explanation could be that the mitosis and interphase II phases accounted for a very small portion of the cell cycle in diploid cells. Thus, G_2 peak was hard to detect because most of the cells were at the stage of interphase I (G_1 or G_0). Haploid cells might have an abnormal cell cycle with a longer cycle time and a relatively larger portion staying at interphase II and mitosis phases. Thus, haploid individuals had a smaller number of cells (smaller and deformed embryos).

Gynogenetic muskellunge were induced by heat shock in most of the experimental groups. A broad range of heat shock conditions (temperature 26–30°C; initial time of heat shock 5–30 min post-fertilization; duration 5–30 min) inhibited the extrusion of the second polar body at a rate of 0.4–6%. Heat shock at 30°C for 8–10 min starting at 20 min post-fertilization appeared to be optimal for induction of chromosome duplication in muskellunge. Luczynski and Woznicki (1995) reported a successful induction of triploids in the northern pike (*Esox lucius*); up to 50% triploids with 50% survival compared with control groups, using a heat shock at 28°C for 10 min starting at 10 min post-fertilization. The survival to hatching in our study was 0.2–3.3% and the highest yield of gynogens was only 6%. It is possible that changes in heat shock conditions could improve the yield of gynogens, but an increased temperature or duration of the heat shock might also result in a high mortality and an increased risk of damage of the genetic material of eggs. Low survival of eggs was found in the muskellunge (our unpublished data) as well as in the northern pike (Luczynski and Woznicki 1995) when eggs were treated at 32°C for retention of the second polar body. Thus, the use of relatively low-intensity heat shock (30°C for 8–10 min) for chromosome duplication remains the preferable option.

The low yield of gynogens and hatching rate does not preclude mass production of gynogenetic muskellunge because the fish has a high fecundity. The abnormal haploid fish are eliminated soon after hatching because they do not survive through yolk-sac absorption. Because gynogenetic fish are not desirable for direct stocking into natural water bodies, a small number of gynogenetic muskellunge females is required for sex reversal and then phenotypic males can be used as broodstock in producing all-female populations.

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Elevated summer temperature effects on megalopal and early juvenile development in the Dungeness crab, *Cancer magister*

S.D. Sulkin, E. Mojica, and G.L. McKeen

Abstract: Commercial fishery stocks of the Dungeness crab, *Cancer magister*, are absent in regions of the middle Georgia Strait, Canada, where suitable benthic habitat occurs and where a plentiful supply of post-larval megalopae is present in nearby waters. This has led to speculation that high summertime temperatures in the region may result in lack of settlement or high juvenile mortality. The present study determined survival, time to metamorphosis, and growth of megalopae exposed to temperatures of 14, 18, and 22°C. Newly metamorphosed juveniles were subjected to the same temperature treatments through the fourth crab instar. Although megalopal survival was not affected by temperature, juvenile crabs showed reduction in growth rate and high mortality in 22°C. Results support the hypothesis that commercial crab fishery stocks in the region are absent because of high mortality of newly settled juveniles where summer water temperatures exceed 18°C.

Résumé : Il n'y a pas de stocks de crabes dormeurs, *Cancer magister*, d'importance commerciale dans la région moyenne du détroit de Georgia (Canada) où l'habitat benthique convient pourtant à cette espèce et où les eaux avoisinantes sont riches en mégalopes post-larvaires. Cet état de choses donne à penser que les températures élevées de l'été qu'on observe dans cette région pourraient expliquer pourquoi il ne s'établit pas de population ou pourraient être la cause d'une forte mortalité juvénile. Dans le cadre de l'étude présentée ici, on a déterminé la survie, l'époque de la métamorphose et la croissance de mégalopes exposées à des températures de 14, 18 et 22°C. Des juvéniles récemment métamorphosés ont été soumis aux mêmes températures pendant le quatrième stade de leur développement. La température n'a pas eu d'influence sur la survie des mégalopes, mais on a constaté une baisse du taux de croissance et une forte mortalité chez les crabes juvéniles élevés à 22°C. Les résultats de cette étude viennent étayer l'hypothèse selon laquelle l'absence de stocks d'importance commerciale dans la région étudiée est due à la forte mortalité des juvéniles qui viennent de s'implanter lorsque la température dépasse 18°C en été. [Traduit par la Rédaction]

Introduction

The Dungeness crab, *Cancer magister*, is fished commercially from central California to Unalaska Island, Alaska. Typical adult habitat includes the shallow sandy areas of the sublittoral zone along the outer coast, although a substantial population and fishery exists within the Puget Sound – Georgia Strait region traversing the Canada – United States border (Fig. 1). This "inland water" population exhibits characteristics of larval size and settlement patterns distinct from those of outer coast populations (Orensanz and Gallucci 1988; DeBrosse et al. 1990; Dinnel et al. 1993; Jamieson and Phillips 1993). Indeed, Jamieson and Phillips (1993) reported differences between Dungeness crab populations inhabiting the west (oceanic) and east (Georgia Strait) coasts of Vancouver Island and identified larval behavioral traits of the inland populations that may restrict their transport and isolate them from outer coast populations.

In the Canadian portion of the inland water habitat, populations abundant enough to support a commercial fishery occur in the southern portion of the Strait of Georgia from the Fraser River estuary south to Boundary Bay (Fig. 1) (Jamieson 1985; Jamieson and Phillips 1993). However, such populations are rare along the east coast of Vancouver Island north of Nanaimo, B.C., even though extensive areas of suitable benthic habitat are present (Jamieson and Phillips 1993). Furthermore, it seems unlikely that the absence of fishery stocks in this middle Georgia Strait region is due to a lack of larvae available for settlement, since adjacent waters contain high densities of post-larval megalopae (Jamieson and Phillips, 1993).

However, in the shallow benthic habitats typically occupied by early juvenile crabs (McMillan et al. 1995), water temperatures in the middle Georgia Strait may exceed 20°C during the late summer (Thomson 1981), the time of greatest occurrence of settlement of inland populations of megalopae (Orensanz and Gallucci 1988; Dinnel et al. 1993; Jamieson and Phillips 1993). Indeed, data from Baynes Sound (Fig. 1) indicated upper temperatures of 22°C in the mid-intertidal waters in 1991–1992 (G. Jamieson, personal communication). By contrast, water temperatures in other regions of the inland water system known to support Dungeness settlement rarely exceed 16°C during the summer (McMillan et al. 1995; personal observation).

Sulkin and McKeen (1989) reported that zoeae raised in the laboratory could not develop successfully to the megalopa at 20°C, although upper temperature limits of megalopae

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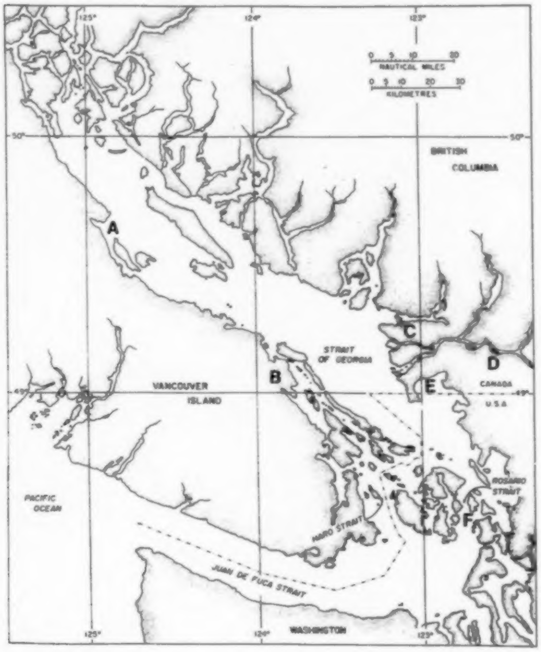

Fig. 1. Inland waters of the northern Puget Sound basin and Georgia Strait. Referenced locations: (A) Baynes Sound, (B) Nanaimo, B.C., (C) Vancouver, B.C., (D) Fraser River, (E) Boundary Bay, (F) Shannon Point Marine Center. Adapted from Jamieson and Phillips (1993, Fig. 3)

have not been reported. Jamieson and Phillips (1993) suggested that larvae in the middle Georgia Strait region may be stressed by exposure to temperature at or exceeding their upper tolerance limits. G. Jamieson (personal communication) further suggests that high shallow-water temperatures in the middle Georgia Strait region could account for the absence of commercial quantities of Dungeness crabs in otherwise suitable habitat.

In the present study, we collected Dungeness crab megalopae from the northern Puget Sound basin and raised them at elevated temperatures to determine their ability to develop successfully to the first juvenile crab stage. We also determined the ability of newly metamorphosed crabs to survive, molt, and grow at the elevated summer temperatures present at otherwise suitable Dungeness crab habitat in the middle Georgia Strait region.

Methods

Approximately 350 *Cancer magister* megalopae were captured at the water surface with a dipnet near the Shannon Point Marine Center, Anacortes, Wash. (Fig. 1). Megalopae were returned to the Marine Center where they were held in large trays fed with running seawater at ambient conditions (29 ppt salinity; 13°C) that were identical to conditions at the nearby capture site. Megalopae were identifed tentatively to species based on size (DeBrosse et al. 1990) and all those used in experiments were confirmed as *C. magister* upon molt to the crab stage.

Effect of temperature on megalopa development

A total of 150 megalopae were selected randomly from the pool and placed in a glass bowl filled with filtered seawater at ambient conditions. Individual megalopae were then assigned randomly to styrene plastic culture boxes measuring 32 × 22 × 4 cm, each culture box containing 24 individual chambers each containing 50 mL of seawater and one megalopa. When all 150 megalopae had been distributed among the culture boxes, the boxes were distributed randomly among the three temperature treatments, each treatment receiving a total of 50 larvae. Investigations in our laboratory on the distribution of intermolt stages among cohorts of megalopae collected locally have indicated that cohort members are generally at the same stage of development when collected. Although it was not possible to identify specific intermolt stages of development of experimental subjects used in this study due to the destructive nature of the procedure (Hatfield 1983), we assumed that megalopae within a cohort collected together were of the same approximate stage of development and that random distribution of individuals among treatments would account for differences that might be present (Forward et al. 1994).

The three temperature treatments used in the study were 14, 18, and 22°C. Megalopae were exposed to higher temperatures (24–30°C) in preliminary experiments; however, high mortality occurred in all cases within 24 h. The lowest temperature used (14°C) is representative of temperatures in most of the inland water region during the late summer when the peak of megalopal settlement occurs (Dinnel et al. 1993; Jamieson and Phillips 1993); whereas 22°C is representative of the highest temperatures reported for the middle Georgia Strait region.

Megalopae were maintained in constant temperature incubators set to the appropriate temperature with a 14 h light : 10 h dark cycle. Megalopae were changed to clean, filtered seawater daily and fed freshly hatched nauplii of the brine shrimp, *Artemia* sp., in excess. Culture water was treated with the antibiotic chloramphenicol (5 mg/L) (Sulkin and McKeen 1989). Each megalopa was examined daily to note incidence of mortality and molting. Megalopae that successfully underwent metamorphosis to the first juvenile crab instar (crab I) were maintained in laboratory culture through the molt to crab II at which time they were rinsed in freshwater, blotted dry, dried in a drying oven on a preweighed aluminum boat for 24 h, and weighed on a Mettler PE 160 analytical balance to the nearest 0.1 mg. Mean time to metamorphosis and mean dry weight of day 1 crab II individuals were compared among treatments.

Effect of temperature on juvenile survival and growth

Megalopae remaining in the original pool of those collected were maintained at 14°C until they metamorphosed to the crab I stage. Megalopae successfully molting to crab I were then randomly distributed among the three temperature treatments. Since not all megalopae molted to crab I on the same day, only day 1 crabs obtained from the mass culture in multiples exceeding three on any one day were used in the experiment. A total of 58 individuals was tested at each of the three temperature treatments. Crabs were maintained individually in the culture boxes following the same procedures as described above. Incidence of mortality and molting was noted daily to determine percent survival and instar duration for crab stages I–IV.

Results

Megalopa development

Although megalopal survival was not directly affected by the temperatures tested, both time to metamorphosis and growth differed among treatments. Survival to metamorphosis was virtually complete and consistent among all temperatures (Table 1). However, as temperature increased, mean time to

Table 1. Percent metamorphosis and mean time to metamorphosis for megalopae raised in the three indicated temperatures.

Temperature treatment (°C)	*n*	% metamorphosis	Time to metamorphosis (days)
14	50	98	7.5 (4.9)*a*
18	50	100	6.7 (4.4)*a*
22	50	98	3.3 (1.6)*b*

Note: Values for time to metamorphosis are means with SDs given in parentheses. Values with the same letter are not significantly different ($P > 0.05$) by nonparametric Tukey's multiple comparison test. *n*, number of megalopae initially exposed to the temperature treatment.

Table 2. Mean weights, with SDs given in parentheses of day 1 crab II individuals in each of the indicated temperatures.

Temperature treatment (°C)	Weight (mg)
14	9.9 (1.5)*a*
18	8.6 (1.7)*b*
22	7.7 (1.3)*c*

Note: Data were analyzed with a parametric one-way ANOVA and a subsequent Tukey pairwise comparison of means. Values with the same letter are not significantly different ($P > 0.05$).

Table 3. Percent survival through successive crab stages at indicated temperatures ($n = 58$ at beginning of experiment for each treatment).

Temperature treatment (°C)	Crab stage I	II	III	IV
14	100	95	93	84
18	98	97	95	90
22	98	81	10	2

metamorphosis decreased (Table 1). Because variances for megalopa duration were unequal (Bartlett's test, $P < 0.05$; Zar 1984), the sample distributions were compared using the nonparametric Kruskal–Wallis ANOVA (Zar 1984), the results indicating significant differences among the samples ($P < 0.001$). Samples were further compared using the nonparametric Tukey's multiple comparison test for unequal sample sizes ($P = 0.05$; Zar 1984). Times to metamorphosis at 14 and 18°C were not significantly different, but both were significantly longer than time to metamorphosis at 22°C (Table 1). Mean weight of day 1 crab II individuals also declined significantly with increasing temperature (Table 2; ANOVA, $P < 0.05$; Tukey's test for pairwise comparisons of means, $P = 0.05$).

Juvenile development

Survival differed among temperatures during the early juvenile stages. A substantial reduction in survival occurred as early as crab II at 22°C, with only 2% surviving the crab IV stage at this temperature (Table 3). This compares with 84 and 90% survival through crab IV at 14°C and 18°C, respectively.

Duration of early juvenile crab instars varied with temperature. Duration of crab I was significantly longer at 14°C than at the two higher temperatures (Table 4; Kruskal–Wallis ANOVA, $P < 0.001$; Tukey's multiple comparison tests, $P = 0.05$). Durations of crab I instars were not significantly different from one another at 18°C and 22°C (Table 4). Significant differences in crab II and crab III instar durations were also found among temperature treatments (Table 4; Kruskal–Wallis ANOVA, $Ps < 0.001$). Results of Tukey's tests ($Ps = 0.05$) for both instars indicated that instar duration at 18°C was significantly shorter than at 14°C. However, instar duration at 22°C was significantly longer than at 18°C but not significantly different from that at 14°C. Instar durations for crab IV were not compared among temperatures because high mortality at 22°C resulted in very small sample size.

Discussion

Although Sulkin and McKeen (1989) reported that zoeae maintained throughout development at 20°C could not develop to the megalopa, and Reed (1969) reported similar results at temperatures as high as 21.7°C, the present study indicates that once the megalopa stage is reached, the postlarvae are tolerant of temperatures as high as 22°C. At 22°C, megalopae showed accelerated development to metamorphosis but smaller size at the crab II stage than was the case for megalopae maintained at lower temperatures. However, it is unlikely that megalopae advected into the middle Georgia Strait will be exposed to high surface temperatures through most of their planktonic period. Jamieson and Phillips (1993) reported that daytime distributions of megalopae were concentrated at depths exceeding 120 m, where the water temperatures were generally less than 10°C. As megalopae entrained in middle Georgia Strait waters approach metamorphosis and move into more shallow regions prior to settlement, however, they are likely to encounter temperatures approaching 22°C. Nevertheless, these results suggest that the relatively high summertime temperatures that megalopae may experience in the middle Georgia Strait shallow water benthic habitat should not prevent successful metamorphosis and settlement. Indeed, it might be argued that accelerated development will promote increased settlement success by reducing the amount of natural mortality due to such factors as predation while the megalopae are still planktonic prior to settlement.

Results of the juvenile study suggest further that high temperatures that occur in the middle Georgia Strait shallow-water benthic environment should have little impact on the first juvenile crab instar, although some reduction in growth may occur. However, by the crab II stage, there is evidence of a significant reduction in growth rate at 22°C and, by the crab III stage, this reduction in growth rate is accompanied by high mortality.

The direct relationship between growth rate and temperature seen in crab I is expected and consistent with results from

Table 4. Mean duration (days), with SDs given in parentheses, of crab stages I–III for juvenile crabs cultured in the three indicated temperatures.

Treatment temperature (°C)	Crab I		Crab II		Crab III	
	n	Duration (days)	*n*	Duration (days)	*n*	Duration (days)
14	58	13.2 (1.9)*a*	55	14.4 (4.3)*a*	52	17.2 (4.4)*a*
18	57	10.5 (2.3)*b*	56	11.5 (2.9)*b*	55	15.3 (5.1)*b*
22	56	9.9 (1.6)*b*	47	15.1 (10.9)*a*	6	25.8 (16.5)*a*

Note: Within each stage, the three temperature treatments were compared by the Kruskall–Wallis ANOVA followed by the nonparametric Tukey's multiple comparison test for unequal sample sizes. For each stage, values with the same letter are not significantly different. *n*, number of juvenile crabs that successfully molted to each crab stage in each temperature treatment.

field studies (e.g., McMillan et al. 1995). That this relationship breaks down as early as crab II, with instar duration becoming longer at 22°C than at 18°C, suggests the presence of temperature-induced stress at the higher temperature. This is supported by the substantial increase in mortality that occurs in the crab III stage at 22°C.

These results suggest that megalopal settlement should be a common phenomenon in suitable benthic habitat along the east coast of Vancouver Island north of Nanaimo, B.C., and that sampling in the region should reveal evidence of crab I and crab II instars. However, the high temperatures that typify the shallow-water benthic habitat during the period when peak settlement and early juvenile growth are occurring could result in high mortality of subsequent juvenile stages. The results are consistent with the hypothesis of Jamieson and Phillips (1993) that the absence of Dungeness crab fishery stocks in the middle Georgia Strait region is not due to failure of larval supply and settlement but to the effects of high temperature on subsequent juvenile survival.

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Assessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induced sac fry mortality in lake trout (*Salvelinus namaycush*) from different regions of the Great Lakes[1]

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Abstract: Background levels of TCDD toxic equivalents (TEs) in lake trout (*Salvelinus namaycush*) eggs (calculated using fish-specific toxicity equivalency factors (TEFs) for polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs)) were higher for two locations in Lake Ontario than Lake Superior and a hatchery (10.5–10.9 versus 0.3 and 0.05 pg TE/g egg). Despite the higher contamination of Lake Ontario eggs, sac fry mortality was uniformly low for all eggs. This is consistent with TCDD toxicity equivalence concentrations (TECs) of all eggs being less than the TCDD no observable adverse effect level (NOAEL) for Lake Superior lake trout sac fry mortality (34 pg TCDD/g egg). Eggs exposed in the laboratory to [^{3}H]TCDD and maintained at either 8 or 8–3–8°C had similar sac fry mortalities. For all sources of eggs, [^{3}H]TCDD-induced sac fry mortality was associated with blue sac disease, which was not affected by water temperature, and resulted in similar LD_{50} values (42–72 pg [^{3}H]TCDD/g egg). Thus, lack of sac fry mortality in wild Lake Ontario lake trout eggs was not caused by lack of responsiveness to TCDD but rather was attributable to egg TECs being below the TCDD NOAEL for sac fry mortality.

Résumé : La concentration naturelle exprimée en équivalents de toxicité (ÉT) de la TCDD dans les oeufs de touladi (*Salvelinus namaycush*) (déterminés à partir des facteurs caractéristiques aux poissons d'équivalence de la toxicité des dibenzo-*p*-dioxines et dibenzofuranes polychlorés (PCDD et PCDF) ainsi que des biphényles polychlorés (BPC), était plus élevée à deux stations du lac Ontario qu'au lac Supérieur et dans une écloserie (10,5–10,9 contre 0,3 et 0,05 pg ÉT/g d'oeufs). Malgré la contamination plus marquée des oeufs du lac Ontario, la mortalité des alevins vésiculés était uniformément faible peu importe la provenance des oeufs. Cette constatation est conforme au fait que la concentration exprimée en équivalents de toxicité (ÉT) de la TCDD dans tous les oeufs est inférieure à la concentration sans effet nocif observé (NOAEL) de cette substance (soit 34 pg TCDD/g d'oeufs) déterminée en termes de mortalité des alevins vésiculés du touladi du lac Supérieur. Le taux de mortalité d'alevins vésiculés issus d'oeufs exposés à la [^{3}H]TCDD au laboratoire et conservés à une température de 8 ou de 8–3–8°C, était similaire. Peu importe l'origine des oeufs, la mortalité causée par la [^{3}H]TCDD chez les alevins vésiculés était associée à la maladie du sac bleu; celle-ci n'était pas soumise à l'influence de la température de l'eau. Cette mortalité correspondait à une CL_{50} comparable, soit de 42–72 pg [^{3}H]TCDD/g d'oeufs. Ainsi, la mortalité réduite des alevins vésiculés issus d'oeufs pondus par des génitrices sauvages du lac Ontario n'est pas attribuable à une réaction peu marqeé à la TCDD, mais plutôt au fait que la concentration des équivalents de toxicité est inférieure à la NOAEL de la TCDD mesurée par la mortalité des alevins vésiculés.
[Traduit par la Rédaction]

Introduction

The Lake Ontario ecosystem has been used extensively as an archetype for studying problems associated with natural reproduction of lake trout (*Salvelinus namaycush*) and for measuring and modeling levels of 2,3,7,8-tetrachlorodibenzo-

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p-dioxin (TCDD) and other persistent halogenated aromatic chemicals in the Great Lakes. Lake Ontario has the most widespread TCDD contamination of all the Great Lakes (U.S. EPA 1990; Cook et al. 1991). Several investigators (O'Keefe et al. 1983; Stalling et al. 1983; DeVault et al. 1989; Zacharewski et al. 1989) have reported the presence of TCDD in several Lake Ontario fish species, with greatest concentrations present in lake trout and other salmonids having a high lipid content. In addition, concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) have been measured in salmonid eggs from the Great Lakes (Niimi 1983; Mac et al. 1985; Smith et al. 1990; Cook et al. 1994). The bioaccumulation of these persistent chemicals in fish early life stages is critical to the characterization of risks of TCDD and related compounds in Lake Ontario for two reasons. First, early life stages of fish are generally more sensitive than adults to toxicity from exposure to TCDD and other Ah receptor agonists (Walker and Peterson 1994). Among all fish species studied thus far lake trout are the most sensitive to sac fry mortality caused by such exposure of eggs. This implies that environmental concentrations of TCDD and related compounds will probably pose the greatest risk to recruitment of sac fry to wild lake trout populations through lethal effects on early life stages. Second, the major route of accumulation of halogenated aromatic chemicals in wild salmonid eggs is through the transfer from maternal tissue during vitellogenesis (Guiney et al. 1979; Niimi 1983; Vodicnik and Peterson 1985; Walker et al. 1994) rather than through uptake from water.

After approximately two decades of progressive lake trout population decline, lake trout in Lake Ontario were essentially extinct by 1960 (Hartman 1988), while contamination by TCDD and various other anthropogenic organochlorine chemicals increased significantly beginning in the 1940s. This initial decline of the lake trout population was attributed to a combination of commercial overfishing, sea lamprey predation, and habitat degradation including eutrophication (Christie 1974). Concentrations of TCDD and related chemicals in wild lake trout eggs, predicted on the basis of the Lake Ontario sediment record, probably caused extensive sac fry mortality during the period of lake trout decline (Cook et al. 1994). Thus, reduced exposure of lake trout to these chemicals is an important requirement for re-establishment of natural reproduction. A lamprey control program was initiated in 1971, and successful stocking of lake trout began in 1973. In 1978, the restoration of a naturally reproducing lake trout population became a Lake Ontario management objective under the Great Lakes Water Quality Agreement (Edwards et al. 1990). Despite extensive restocking (in excess of 2 million lake trout annually since 1985) and the successful re-establishment of adult lake trout in Lake Ontario, the evidence for a naturally reproducing population remains limited. Since 1984, Lake Ontario lake trout eggs have been successfully propagated in U.S. Fish and Wildlife Service hatcheries for the subsequent stocking of yearlings (Marsden et al. 1988; Elrod et al. 1988). Although the data on the percentage of blue sac disease and other early life stage mortality are not reported in these studies, they suggest that at least since 1984, the contaminant load contained within these eggs does not appear to directly affect egg viability. The first report of natural reproduction since stocking began in 1973 was by Marsden et al. (1988). Since 1986, only a few other investigators have reported observing fry emergence (Marsden and Krueger 1991; Grewe et al. 1994) or collecting wild indigenous yearling lake trout (Casselman 1991). This suggests that naturally produced lake trout sac fry or fry have experienced mortality that contributed to recruitment failure of lake trout in Lake Ontario since 1984.

Early life stage mortality due to blue sac disease, a naturally occurring edematous condition observed in hatchery-raised salmonid sac fry (Wolf 1969; Roberts and Shepherd 1986), has been documented in Lake Ontario lake trout (Symula et al. 1990; Simonin et al. 1990). A strong relationship has also been established between TCDD exposure of lake trout eggs via waterborne exposure (Spitsbergen et al. 1991; Walker et al. 1991), injection (Walker et al. 1992), and maternal transfer (Walker et al. 1994), and blue sac syndrome associated mortality of sac fry. Significant mortality during lake trout early life stage development has also been documented from various other regions of the Great Lakes, and the presence of PCDDs, PCDFs, and (or) PCBs in wild lake trout eggs has been proposed as one explanation for this mortality and natural recruitment failure (Willford et al. 1981; Eschenroder et al. 1984; Mac et al. 1985, 1988; Symula et al. 1990; Walker and Peterson 1990).

Cook et al. (1994) have estimated that TCDD concentrations in Lake Ontario lake trout eggs declined approximately 65% between 1978 and 1988. This is consistent with the relatively high (48%) incidence of blue sac disease and associated mortality reported by Symula et al. (1990) for hatchery reared lake trout eggs collected in 1979 from Lake Ontario. Even at reduced egg concentrations, TCDD in combination with other TCDD-like PCDDs, PCDFs, and PCBs may still contribute sufficient toxicity to act in concert with other environmental stressors to impede the re-establishment of naturally reproducing lake trout populations.

This study had three major objectives. The first was to determine if the hallmark sign of TCDD-induced early life stage toxicity, blue sac syndrome, was occurring in fertilized lake trout eggs obtained from Lake Ontario in 1991. Secondly, this study sought to determine if Lake Ontario lake trout sac fry, which were naturally exposed to TCDD and related chemicals, would display a similar sensitivity to [^{3}H]TCDD induced early life stage mortality compared with hatchery-reared and wild Lake Superior lake trout sac fry contaminated with lower levels of these chemicals. To this end, background concentrations of PCDD, PCDF, and PCB congeners that have been shown to produce sac fry mortality associated with a blue sac syndrome were determined in lake trout eggs from eastern and western Lake Ontario, Lake Superior, and from a hatchery, using high resolution gas chromatography – high resolution mass spectrometry (HRGC–HRMS). Background mortality due to blue sac syndrome was determined in the wild and hatchery lake trout sac fry along with their response to graded egg doses of [^{3}H]TCDD. In addition, sac fry from Lake Ontario were observed for a period of 1 month past swim-up to assess the incidence of "swim-up syndrome," an effect not known to be associated with TCDD exposure in salmonids but which occurs in lake trout sac fry from Lake Michigan (Mac et al. 1993) and Lake Ontario (Fitzsimons et al. 1995). Because water temperature may modulate the occurrence and severity of blue sac disease in salmonids (Ostergaard, 1987), our third objective was to determine if the sensitivity of lake trout sac fry to TCDD-induced mortality by blue sac syndrome would be affected if the study were conducted at a constant water temperature of 8°C versus a water temperature profile that lake trout eggs, embryos, sac fry, and fry might typically encounter in the environment (i.e., a variable temperature of 8–3–8°C). The 8–3–8°C temperature profile was designed to resemble the seasonal water temperature fluctuations anticipated at lake trout spawning reefs in the Great Lakes (Martin and Olver 1980; Bronte 1993; Casselman 1995).

Materials and methods

[^{3}H]TCDD

[1,6-^{3}H]TCDD was obtained from Chemsyn Science (Lenexa, Kan.) and purified to a final radiochemical purity of >99% (specific activity 33.6 Ci/mmol (1 Ci = 37 GBq)) by reverse-phase high performance liquid chromatography (HPLC) as described by Olson (1986). TCDD was stored in HPLC-grade acetone (Aldrich, Milwaukee, Wis.) at −20°C prior to dilution as stock solutions in anhydrous 1,4-*p*-dioxane (Aldrich).

Lake trout egg collection

Lake trout eggs were obtained from wild populations collected by gill nets at two distinct Lake Ontario spawning shoals during October–November 1991. Eggs were stripped from 6 females from Fifty Point in western Lake Ontario and 12 females from Stony Island in eastern Lake Ontario and fertilized with the milt of multiple males at each respective location. Eggs were similarly collected from 28 females and fertilized with milt from multiple males off Gull Island Shoal in southwestern Lake Superior. These eggs were used as a reference site for comparing sensitivity to TCDD-induced sac fry mortality. In addition, fertilized lake trout eggs were also obtained from Crystal Springs hatchery (Altura, Minn.) to serve as a control group. After water hardening and within 24 h of fertilization the eggs from each of the locations were transported to Madison, Wis.

Background contaminant concentrations in lake trout eggs and calculation of fish-specific TECs in lake trout eggs

Concentrations of individual PCB, PCDF, and PCDD congeners in fertilized lake trout eggs from all sources were analyzed. Egg samples (10.1 ± 0.1 g wet weight; mean ± SD) were ground, homogenized, and blended with anhydrous sodium sulfate (50 g). After addition of ^{13}C$_{12}$-labeled surrogate standards for PCDDs and PCDFs (Marquis et al. 1994) and PCBs (Kuehl et al. 1991), the samples were Soxhlet extracted with hexane – methylene chloride (1:1, v:v) for 12 h. Lipids, cholesterol, fatty acids, and other interferences were removed through gel permeation chromatography followed by 1% deactivated silica gel column chromatography. PCDDs, PCDFs, and PCBs in the purified extracts were isolated from more polar chemicals through high pressure liquid chromatography (HPLC) using a cyanopropyl (Phase-Sep, UK) semipreparative column in normal phase with a hexane – methylene chloride solvent gradient. Further HPLC treatment of the nonpolar fraction containing the analytes of interest was accomplished with a pyrenyl column (Nacalai Tesque, Japan) in normal phase with a hexane–toluene gradient, which resulted in three fractions for HRGC–HRMS analysis: PCDDs and PCDFs, non-ortho-chlorinated biphenyls, and ortho-chlorinated biphenyls. Concentrations of individual PCB, PCDF, and PCDD congeners in lake trout eggs were analyzed with a Finnigan-Mat 8230 double-focusing HRGC–HRMS instrument using a 30-m DB-5 gas chromatography column. The HRGC–HRMS conditions, quantification procedures, and quality assurance criteria have been described previously (Marquis et al. 1994), with the exception that minimum levels of detection for this study were calculated on the basis of a 3:1 signal to noise criteria for each analyte in each sample.

The "background" TCDD toxicity equivalence concentrations (TECs) due to PCBs, PCDDs, and PCDFs in eggs from each source was estimated. For Lake Superior lake trout eggs from Gull Island Shoal, concentrations of each chemical measured in three annual samples of eggs collected during 1989–1991 were averaged for the TEC calculation. Trout-specific TCDD toxicity equivalence factors (TEFs) for each chemical based on the molar concentration of each chemical in the egg that causes 50% mortality in rainbow trout (*Oncorhynchus mykiss*) sac fry (Walker and Peterson 1991; Zabel et al. 1995*a*) were used. The TCDD toxicity equivalents (TE$_x$) due to each chemical (x) in the eggs was calculated as

$$\mathrm{TE}_x = \mathrm{TEF}_x(\mathrm{MW}_{\mathrm{TCDD}}/\mathrm{MW}_x)\ \mathrm{Cegg}_x$$

where MW is the molecular weights used to convert TEFs from molar to mass concentration units and Cegg is the concentration of chemical measured in eggs as picograms per gram wet mass of eggs. The sum of TEs for each chemical in an egg sample equals the TEC. The units of TEs and TEC are picograms TCDD equivalent per gram wet mass of eggs. The additive TE model has been validated for trout early life stage mortality with binary mixtures of congeners in rainbow trout eggs (Zabel et al. 1995*b*), TCDD and PCB 126 in lake trout eggs (Zabel et al. 1995*c*), and a complex mixture of 14 congeners in lake trout and rainbow trout eggs (Walker et al. 1996).

Static water exposure of lake trout eggs to [^{3}H]TCDD

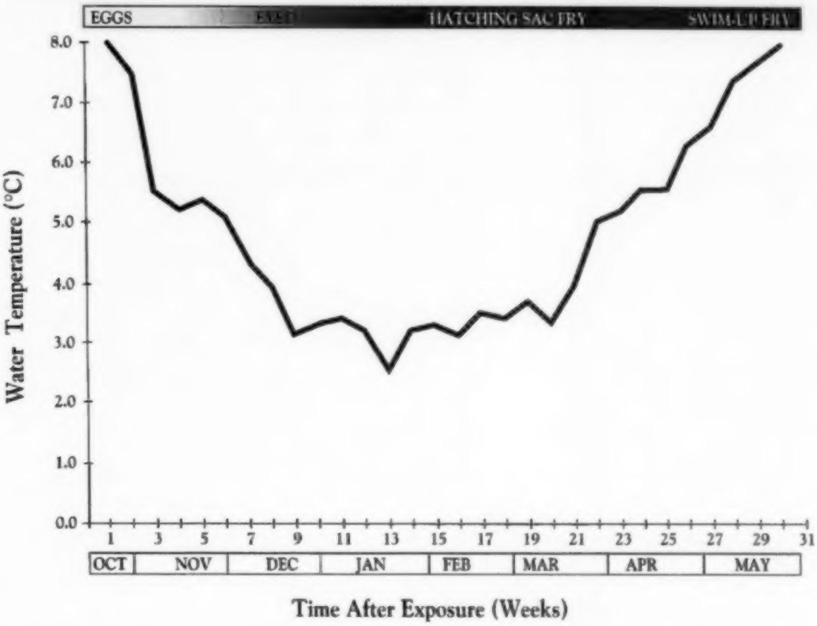
Eggs from each source were treated prophylactically with 100 ppm Argentyne disinfectant (Argent Laboratories, Redmond, Wash.) for 10 min before they were divided into subgroups; egg numbers were estimated by volume displacement. Lake Ontario and hatchery eggs were divided into 32 subgroups (200 eggs per subgroup), which were randomly assigned to 1 of 16 treatment groups. The 16 groups consisted of 2 vehicle control groups and 14 graded [^{3}H]TCDD dosage groups. Fertilized Lake Superior eggs were divided into 24 subgroups (1300 eggs per subgroup) that were assigned to 1 of 6 treatment groups, which consisted of a vehicle control and 5 [^{3}H]TCDD dosage groups.

Within 24 h after fertilization, each subgroup of eggs was statically exposed for 48 h to water containing 4 mL acetone/L (solvent control) or [^{3}H]TCDD dissolved in 4 mL acetone/L. For Lake Ontario and hatchery eggs, 2 vehicle controls and 14 nominal water concentrations of [^{3}H]TCDD were used: 2, 4, 6, 8, 10, 15, 20, 30, 40, 60, 80, 100, 120, and 150 ng [^{3}H]TCDD/L water. For Lake Superior eggs, one vehicle control and five nominal water concentrations of [^{3}H]TCDD were used: 10, 20, 40, 62 and 100 ng [^{3}H]TCDD/L water. Throughout the static exposure, the exposure water (6–8°C) was aerated and renewed three times at approximately 12-h intervals with water containing the appropriate concentration of acetone or [^{3}H]TCDD in acetone. Following the 48 h exposure, each group was transferred to an individual tank supplied with flowing [^{3}H]TCDD-free water.

Determination of TCDD-derived [^{3}H] in eggs

To determine the concentration of [^{3}H]TCDD accumulated in lake trout eggs exposed to waterborne [^{3}H]TCDD, five eggs per group were sampled 10 days postexposure (to allow for desorption of [^{3}H]TCDD from the outer surface of the chorionic membrane). Previous work has shown that during the egg and sac fry stages of lake trout development, the absolute amount of [^{3}H]TCDD in eggs and sac fry remains essentially constant with no significant elimination until after swim-up (Walker et al. 1991; Zabel et al. 1995). Lake trout eggs (140–160 mg) were blotted to remove excess water, weighed, and digested and dissolved with 2 mL Soluene 350 (Packard Instrument Co., Downers Grove, IL) at 50–60°C overnight. The

Fig. 1. Average weekly (measured 4 days/week) water temperatures used to maintain lake trout early life stages over the course of this study for comparison with lake trout reared at a constant 8°C.



digested and dissolved samples were cooled, 10 mL of Hionic (Packard) liquid scintillation medium was added, and low-energy chemiluminescence was allowed to decay prior to determining counts per minute (cpm) of TCDD-derived ^{3}H by liquid scintillation counting (Packard, model 2000 CA). Using appropriate quench corrections, disintegrations per minute (dpm) were calculated from cpm and counting efficiency.

Maintenance and assessment of eggs, sac fry, and fry

Eggs, sac fry, and fry were maintained in filtered (rust and sediment type filter), dechlorinated tap water (8–12 replacements/day, dissolved oxygen 12.0 ppm, pH 7.5, hardness 304 ppm as $CaCO_3$, alkalinity 374 ppm as $CaCO_3$). Each group of lake trout eggs was maintained in water kept at 8.0 ± 1.0°C. In addition, duplicate sets of vehicle and [^{3}H]TCDD-exposed eggs from Fifty Point and Stony Island, Lake Ontario, and a hatchery were also maintained under a variable cold water temperature regime (8–3–8°C) designed to more closely mimic seasonal variations in water temperatures on Lake Ontario spawning reefs (Fig. 1). The original experiment included a temperature profile based on expert input from the joint authors of this research. Subsequent findings by Casselman (1995) confirmed the appropriateness of the selected natural water temperature simulation regime. To ensure the appropriate water temperatures, beakers containing each of the egg dose groups were individually supplied with flow-through water and placed in a 38-L glass aquarium (two aquaria/experiment), which contained flow-through water at either a constant 8°C or the 8–3–8°C spawning reef water temperature appropriate for that time of year.

Beginning at swim-up, control and TCDD-treated fry derived from Lake Ontario eggs and maintained at water temperatures of 8–3–8°C were fed No. 1 Rangen trout pellets daily for 14–20 days and then switched to No. 5 Rangen trout pellets ad libitum until termination of the experiment, 1 month after swim-up. The purpose of this experiment was to assess the remaining fry for evidence of "swim-up" or "drop-out" syndrome, which is separate and distinct from blue sac syndrome caused by TCDD, and is reported to occur in a female-specific fashion in Lake Michigan and Lake Ontario lake trout fry (Symula et al. 1990; Simonin et al. 1990). In this study we used the following signs associated with drop-out – swim-up mortality: loss of equilibrium, erratic swimming, fry lying on their sides on the bottom of the tank, and hyperexcitability followed by lethargy prior to death to measure the incidence of this effect.

To assess cumulative mortality throughout these experiments, dead sac fry or fry from each treatment group were removed and recorded every other day. At the termination of each experiment (swim-up or 1 month after swim-up), surviving fry in each treatment group were counted and killed. Cumulative mortality was calculated excluding the viable eggs that were sampled for accumulated [^{3}H]TCDD.

Statistical procedures

Cumulative mortality data at swim-up for each [^{3}H]TCDD treatment group were analyzed by chi-square (Wonnacott and Wonnacott 1985) to determine TCDD-related effects. A continuous dose–response curve was generated for mortality data from hatching onset to swim-up as a function of the egg [^{3}H]TCDD dose using a probit analysis procedure, which accounted for control mortality, used chi-square goodness of fit, estimated the slope and intercept, and was based on the assumption that mortality was independent for fish within a treatment group and among treatment groups (Finney 1971; SAS 1988). The LD_{50} of TCDD (50% mortality above vehicle control group from hatching onset to swim-up) and 95% fiducial limits were calculated based upon the [^{3}H]TCDD concentration accumulated in the eggs.

Results

Background concentrations of PCBs, PCDFs, and PCDDs and calculation of TCDD toxic equivalence concentrations (TECs) in lake trout eggs

Concentrations of PCDD and PCDF congeners in both Lake

Table 1. Concentrations of toxicologically important PCDD, PCDF, and PCB congeners measured in feral Lake Superior lake trout eggs (1989–1991) and in hatchery and feral Lake Ontario lake trout eggs collected in 1991.

	Egg congener concentration (pg/g)			
	Southwestern Lake Superior (Gull Island)[a]	Hatchery (Crystal Springs)	Western Lake Ontario (Fifty Point)	Eastern Lake Ontario (Stony Island)
PCDDs				
2,3,7,8-TCDD	nd (0.2)[b,c]	nd (0.2)	6.9 (1.2)	6.0 (0.7)
1,2,3,7,8-PeCDD	nd (1.1)	nd (0.9)	nd (1.3)	nd (0.8)
1,2,3,4,7,8-HxCDD	nd (0.7)	nd (0.8)	nd (1.3)	nd (0.8)
1,2,3,6,7,8-HxCDD	nd (0.7)	nd (0.8)	nd (1.3)	nd (0.8)
1,2,3,4,6,7,8-HpCDD	nd (0.7)	nd (0.8)	nd (1.1)	nd (1.0)
PCDFs				
2,3,7,8-TCDF	2.1 (0.5)	nd (0.7)	3.9 (0.5)	4.4 (0.8)
1,2,3,7,8-PeCDF	nd (0.6)	nd (0.3)	1.1(1.0)	1.4 (1.1)
2,3,4,7,8-PeCDF	nd (0.6)	nd (0.3)	4.6(1.1)	5.0 (1.2)
1,2,3,4,7,8-HxCDF	nd (0.6)	nd (0.3)	nd (2.2)	1.7 (0.7)
Non-Ortho PCBs				
3,4,4′,5-TCB (No. 81)	na[d]	na[d]	50[e]	50[e]
3,3′4,4′-TCB (No. 77)	54	30	1 000	960
3,3′,4,4′,5-PeCB (No. 126)	52	8	400	380
3,3′,4,4′,5,5′-HxCB (No. 169)	nd (10)	nd (10)	20	16
Mono-Ortho PCBs				
2,3,3′,4,4′-PeCB (No. 105)	800	200	11 000	12 000
2,3,4,4′,5-PeCB (No. 118)	8 000	2 200	39 000[e]	39 000[e]

[a] Concentrations of congeners in Lake Superior, Gull Island Shoal lake trout eggs are mean values from samples collected over the period 1989–1991.

[b] nd, concentration below the limit of detection.

[c] Numbers in parentheses are minimum levels of detection for each congener in the sample.

[d] na, PCB congener was not analyzed when the sample set was processed.

[e] PCB Nos. 81, 105, and 118 concentrations in Lake Ontario lake trout eggs in 1991 have been extrapolated from HRGC–HRMS analysis of 1988 samples.

Superior and hatchery samples were below the HRGC–HRMS limits of detection with the exception of detectable 2,3,7,8-tetrachlorodibenzofuran in the Lake Superior eggs (Table 1). These results are consistent with PCDD and PCDF concentrations previously reported for 1984 Lake Superior lake trout (DeVault et al. 1989) if one considers the approximately 3:1 ratio observed between concentrations in Great Lakes wild lake trout as compared with their eggs (Miller 1993; Cook et al. 1994) and the expected decline in residues between 1984 and 1990. Concentrations of PCDD and PCDF congeners in the lake trout eggs from Lake Ontario are similarly consistent with the concentrations reported for 1984 Lake Ontario lake trout by DeVault et al. (1989).

PCB congeners were detectable in all egg samples, but much larger concentrations were found in those from Lake Ontario. Concentrations of PCB No. 126, the most toxic PCB congener, were 6-fold greater in Lake Superior eggs and 50-fold greater in Lake Ontario eggs than in hatchery eggs. The other PCB congeners were similarly distributed with the exception of PCB No. 77 concentrations, which seemed greater in the hatchery eggs and less in the Lake Superior eggs than expected from the PCB No. 126 distribution.

The TEC for hatchery lake trout eggs was ≥0.05 pg TCDD toxicity equivalent/g wet egg and two PCB congeners (Nos. 118 and 126) contributed >83% of the TECs (Table 2). TECs in lake trout eggs from Lake Superior was ≥0.3 pg TCDD toxicity equivalent/g of which 2,3,7,8-TCDF plus PCBs No. 118 and No. 126 accounted for >96%. TECs were significantly higher in lake trout eggs collected from Lake Ontario. For eggs from Fifty Point and Stony Island, Lake Ontario, TEs contributed by 2,3,7,8-TCDD were predominant, making up 62 and 56% of the TECs, respectively. For lake trout eggs from Fifty Point, PCDFs contributed 16% and PCBs, 22% of the TECs. For Stony Island eggs, PCDFs accounted for 23% and PCBs, 21% of the total TEC.

Sac fry mortality

Sac fry mortality due to blue sac syndrome in vehicle-exposed groups was less than 20% in eggs from all sources (Table 3). For eggs obtained from Lake Ontario, sac fry mortality tended to be higher at 8°C than at 8–3–8°C, whereas the opposite was observed for background sac fry mortality in eggs obtained from the hatchery.

The cumulative mortality of lake trout embryos was unaffected by [^{3}H]TCDD exposure prior to the onset of hatching (data not shown). There was in fact no significant difference in mean egg mortalities between the vehicle control groups and the combined TCDD treatment groups for each source of

Table 2. TCDD toxicity equivalents (TEs) derived from fish-specific toxic equivalency factors (TEFs) based on early life stage mortality and the chemical concentrations measured in hatchery and Great Lakes lake trout eggs as shown in Table 1.

		Egg TEs (pg TCDD equivalents/g wet egg) contributed by individual congeners			
Congener	Fish-specific TEF[a]	Southwestern Lake Superior (Gull Island Shoal)	Hatchery (Crystal Springs)	Western Lake Ontario (Fifty Point)	Eastern Lake Ontario (Stony Island)
PCDDs					
2,3,7,8-TCDD	1.0	nd (0.2)[b,c]	nd (0.2)	6.90	6.00
1,2,3,7,8-PeCDD	0.730	nd (1.1)	nd (0.9)	nd (1.3)	nd (0.8)
1,2,3,4,7,8-HxCDD	0.319	nd (0.7)	nd (0.8)	nd (1.3)	nd (0.8)
1,2,3,6,7,8-HxCDD	0.024	nd (0.7)	nd (0.8)	nd (1.3)	nd (0.8)
1,2,3,4,6,7,8-HpCDD	0.002	nd (0.7)	nd (0.8)	nd (1.1)	nd (1.0)
PCDFs					
2,3,7,8-TCDF	0.028	0.05	nd (0.7)	0.11	0.12
1,2,3,7,8-PeCDF	0.034	nd (0.6)	nd (0.3)	0.04	0.05
2,3,4,7,8-PeCDF	0.359	nd (0.6)	nd (0.3)	1.65	1.80
1,2,3,4,7,8-HxCDF	0.280	nd (0.6)	nd (0.3)	nd (2.2)	0.48
Non-Ortho PCBs					
3,4,4′,5-TCB (No. 81)	0.000 56	na[d]	na[d]	0.03	0.03
3,3′4,4′-TCB (No. 77)	0.000 16	0.009	0.005	0.16	0.15
3,3′,4,4′,5-PeCB (No. 126)	0.005	0.260	0.040	2.000	1.900
3,3′,4,4′,5,5′-HxCB (No. 169)	0.000 041	nd (10)	nd (10)	0.001	0.001
Mono-Ortho PCBs					
2,3,3′,4,4′-PeCB (No. 105)	<0.000 002 6	<0.002	<0.001	<0.029	<0.031
2,3,4,4′,5-PeCB (No. 118)	<0.000 004 5	<0.036	<0.010	<0.176	<0.176
TEC[e]		≥0.32	≥0.05	≥10.89	≥10.53

[a] TEF was calculated by dividing the LD_{50} of 2,3,7,8-TCDD (pmol TCDD/g egg) by the LD_{50} of the congener (pmol congener/g egg) based on early life stage mortality in the same strain of rainbow trout (Walker and Peterson 1991; Zabel et al. 1995*a*).

[b] nd, concentration below the limit of detection.

[c] Numbers in parentheses are minimum limits of analytical detection (pg/g sample) for each congener in the sample.

[d] na, PCB congener was not analyzed when the sample set was processed.

[e] Toxicity equivalence concentrations (TECs) were obtained for each location by summing the individual TCDD toxicity equivalents (TEs) of each PCDD, PCDF, and PCB congener using fish-specific TEF values for each congener that were based on the endpoint of sac fry mortality in rainbow trout (Walker and Peterson 1991; Zabel et al. 1995*a*). The TEs listed for the mono-ortho-PCBs were not included in the final TEC calculations based upon a lack of early life stage mortality as indicated by their TEF values. The ≥ sign in front of each TEC value indicates that the TEC may be greater because of either undetected chemicals or undetected toxicity for some of the chemicals tested.

Table 3. Background sac fry mortality for lake trout eggs collected from various Great Lakes locations and a hatchery source maintained under two different water temperature regimes.

	Lake trout sac fry mortality (%)[a]	
Egg source	8°C	8–3–8°C
Southwestern Lake Superior (Gull Island)	11	nc[b]
Hatchery (Crystal Springs, Altura, Minn.)	6	18
Western Lake Ontario (Fifty Point)	11	7
Eastern Lake Ontario (Stony Island)	8	1

[a] Mean percent cumulative mortality (half hatched plus blue sac syndrome) determined from the onset of hatching to swim-up for two vehicle treatment groups (i.e., non-[^{3}H]TCDD exposed controls).

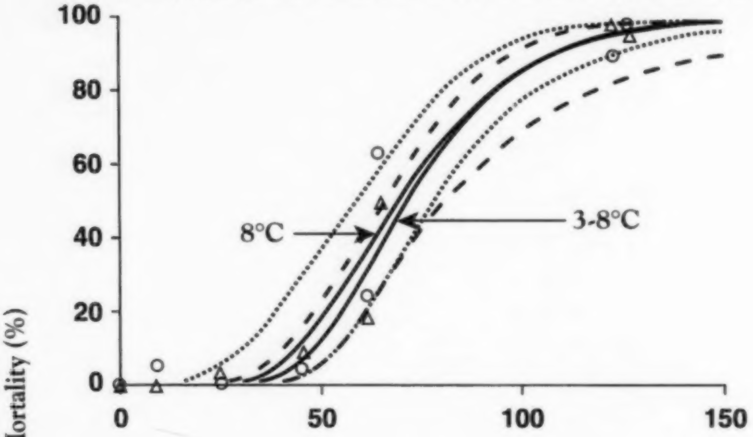
[b] nc, not conducted.

eggs. Furthermore, reducing the incubation temperature from a constant 8°C to the variable 8–3–8°C temperature profile had no effect on egg mortality prior to the onset of hatching.

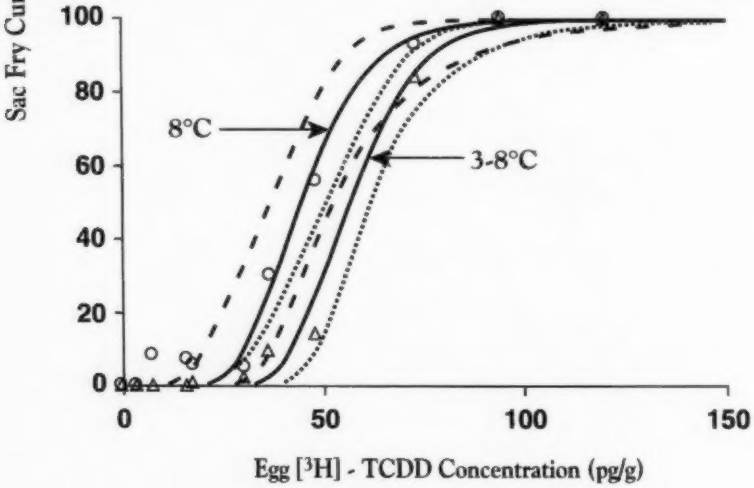
Waterborne exposure of fertilized lake trout eggs to [^{3}H]TCDD resulted in egg [^{3}H]TCDD dose-related increases in cumulative mortality of larvae from the onset of hatching to swim-up. In general, the greatest mortality occurred as sac fry, with less mortality associated with embryos dying partially emerged from the chorion (half-hatched). In sac fry from all sources the signs of TCDD toxicity during the sac fry stage of development were the same. They consisted of subcutaneous hemorrhages, subcutaneous edema of the yolk sac, pericardial and meningeal edema, craniofacial malformations, regional ischemia, and growth retardation, culminating eventually in death during the sac fry stage of development (Spitsbergen et al. 1991; Walker et al. 1991). These signs are essentially identical to those of blue sac disease (Wolf 1969;

Fig. 2. Cumulative mortality of lake trout sac fry from (*a*) Fifty Point and (*b*) Stony Island from hatching onset to swim-up as a function of [^{3}H]TCDD concentrations in eggs incubated at a constant 8°C or 8–3–8°C. Egg [^{3}H]TCDD concentrations shown are based on the accumulated TCDD-derived [^{3}H] measured in eggs 10 days postexposure. The values in parentheses are the background TECs determined for eggs from each location. Cumulative mortality in TCDD groups has been corrected for mean cumulative mortality in the acetone vehicle control group ((○), 8°C mortality data points; (Δ), 8–3–8°C data). Broken lines (8°C) and dotted lines (8–3–8°C) represent the probit model's upper and lower 95% fiducial limits for each probit generated dose–response curve.

a) Fifty Point, Lake Ontario (10.9 pg TCDD toxicity equivalent/g wet egg)



b) Stony Island, Lake Ontario (10.5 pg TCDD toxicity equivalent/g wet egg)

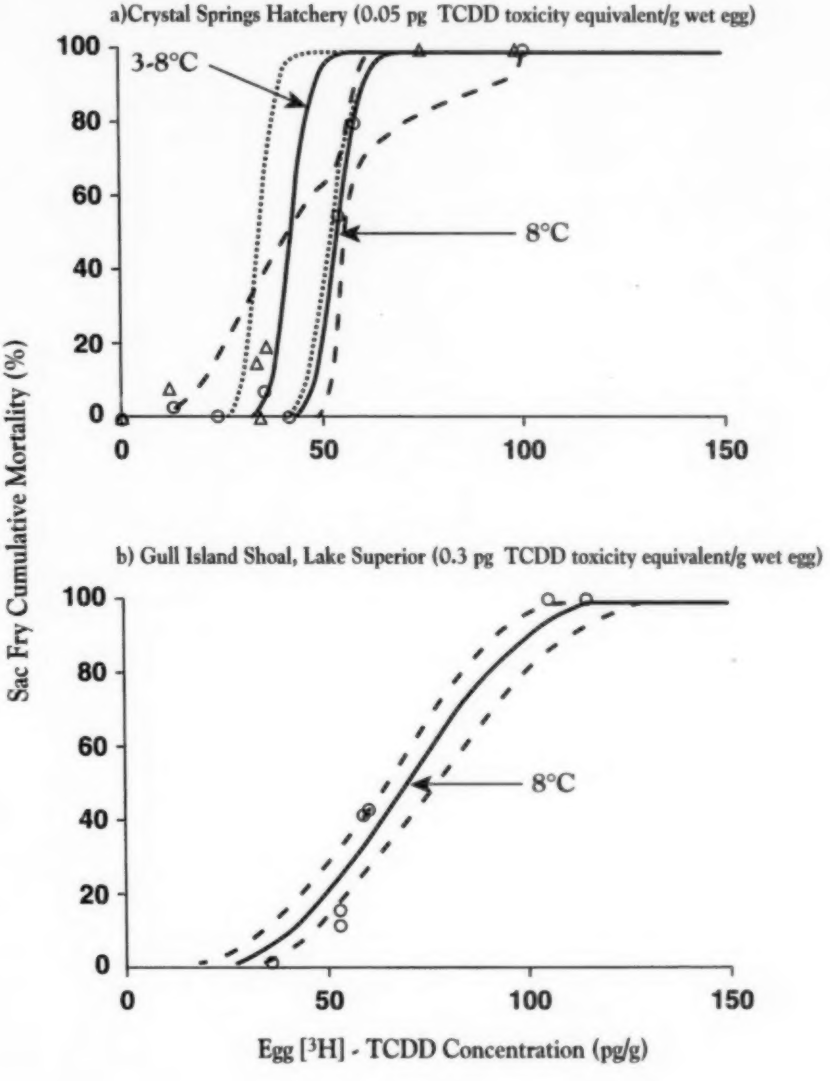


Roberts and Shepherd 1986), which has been reported in the past in Lake Ontario sac fry (Symula et al. 1990; Simonin et al. 1990). For eggs collected from Fifty Point and Stony Island locations and incubated using the 8–3–8°C water temperature profile for up to 1 month after swim-up, the incidence of swim-up syndrome characteristics and associated mortality was low. The average occurrence of swim-up syndrome across all groups ([^{3}H]TCDD exposure groups and control) was 5–8% and was unaffected by [^{3}H]TCDD egg dose or incubation temperature.

TCDD dose response relationships for sac fry mortality

There was no significant effect of water temperature on [^{3}H]TCDD-induced sac fry mortality in eggs obtained from either Fifty Point (Fig. 2*a*) or Stony Island, Lake Ontario (Fig. 2*b*). At both sites and at both temperatures there was a

Fig. 3. Probit-generated dose–response curves for percent cumulative sac fry mortality from hatching onset to swim-up for (*a*) Crystal Springs Hatchery lake trout eggs, exposed to waterborne [^{3}H]TCDD and incubated under two different temperature regimes. Individual mortality data points for 8°C (○) and 8–3–8°C (Δ) have been corrected for control mortality. The probit model's 95% fiduciary limits for each curve are shown by broken lines (8°C) or dotted lines (8–3–8°C). (*b*) Gull Island Shoal lake trout eggs incubated at a constant 8°C (adapted with permission from Walker et al. 1991). The probit model (solid line) is illustrated with the 95% fiducial limits shown as broken lines on either side.



steep [^{3}H]TCDD dose-related increase in lake trout sac fry mortality. The 95% confidence intervals for the egg [^{3}H]TCDD doses at all levels of sac fry mortality at the constant temperature (8°C) overlapped those for the spawning reef temperatures (8–3–8°C).

Essentially the same profile of [^{3}H]TCDD dose-related effects was observed for lake trout reared from hatchery eggs (Fig. 3*a*). In this case the dose–response curve was even steeper than for wild eggs, but again there was no effect of water temperature on the slope of the curve. The dose–response curve for cumulative mortality of Lake Superior Gull Island Shoal sac fry as a function of [^{3}H]TCDD dosage in the egg at a constant 8°C water temperature (Fig. 3*b*) was generally very similar to those observed for wild Lake Ontario lake trout (Fig. 2).

For all sources of eggs the background TECs, which ranged from 0.05 to 10.9 pg TE/g egg, were below the NOAEL for lake trout sac fry mortality (Table 4). The

Table 4. Calculated early life stage toxicity endpoints based on sac fry mortality of lake trout exposed as fertilized eggs to graded concentrations of [^{3}H]2,3,7,8-TCDD and incubated under two different water temperature regimes.

		Egg [^{3}H]TCDD concentration (pg [^{3}H]TCDD/g)			
	Background egg TEC (pg TE/g)	NOAEL[a]		LD$_{50}$[b]	
Egg source		8°C	8–3–8°C	8°C	8–3–8°C
Western Lake Ontario	10.9	45	45	69	72
(Fifty Point)		(31–55)	(37–51)	(58–80)	(65–78)
Eastern Lake Ontario	10.5	30	36	44	57
(Stony Island)		(20–37)	(25–43)	(36–52)	(50–61)
Southwestern Lake Superior	0.3	34	nc[c]	65	nc[c]
(Gull Island)		(26–40)		(60–71)	
Hatchery (Crystal Springs,	0.05	41	35	53	42
Altura, Minn.)		(8–47)	(28–44)	(41-55)	(33-52)

[a] The no observable adverse effect level (NOAEL) was the whole egg [^{3}H]TCDD concentration that did not significantly increase cumulative sac fry mortality compared with control. Values in parentheses are 95% confidence intervals.

[b] LD$_{50}$ values represent TCDD concentrations in eggs that caused 50% mortality above control from hatching onset to swim-up as determined using the probit procedure (Finney 1971; SAS Institute Inc. 1988). Values in parentheses are 95% confidence intervals.

[c] nc, not conducted.

background TEC was, however, higher for eggs from Lake Ontario than from Lake Superior or a hatchery (Table 4). The background TEC in the most heavily contaminated eggs (Fifty Point Lake Ontario) was less than the 95% confidence interval of the LD$_{50}$ determined for the lake trout sac fry in this study. Therefore, it was not surprising that the LD$_{50}$ for sac fry mortality, based on egg [^{3}H]TCDD dose, was generally similar for eggs with low (Lake Superior and hatchery) and higher (western and eastern Lake Ontario) background TECs.

The LD$_{50s}$, based on [^{3}H]TCDD egg dose, ranged from 44 to 69 pg/g for Lake Ontario, Lake Superior, and hatchery sac fry reared at 8°C. For sac fry from those same sources but reared at a water temperature of 8–3–8°C, the range was essentially the same (42–72 pg/g). The range in no observable adverse effect levels (NOAELs) for sac fry mortality, based on egg [^{3}H]TCDD dose, for the same four sources of eggs, was relatively narrow. For sac fry reared at 8°C the NOAEL ranged from 30 to 45 pg/g. At a water temperature of 8–3–8°C, the NOAELs ranged from 35 to 45 pg/g. Taken together, these results demonstrate that water temperatures that would be encountered by lake trout embryos and sac fry at spawning reefs in Lake Ontario would not result in a change in the potency of TCDD in causing sac fry mortality.

Discussion

Relationships between background TECs in Lake Ontario lake trout eggs and background sac fry mortality

Several studies have documented that lake trout eggs collected from Lake Ontario females exhibited high incidences of blue sac disease and associated mortality between 1977 and 1983. In 1977, Simonin et al. (1990) reported a range from 7 to 100% with a mean incidence of 70%. Lake Ontario lake trout eggs collected in 1979 displayed a range of blue sac disease from 2 to 98% with a mean incidence of 48% (Symula et al. 1990). By 1983 these same researchers were reporting a range of blue sac disease incidence for Lake Ontario lake trout of 16–23%. In these studies, lake trout sac fry edema and mortality was female dependent, occurred at a constant 8°C holding temperature, and was not correlated with concentrations of DDT, DDE, total PCBs, dieldrin, or mirex in the eggs. A few years later, Ostergaard (1987) reported a rough estimate of 60% loss of Stony Island Lake Ontario lake trout sac fry due to blue sac disease from eggs collected and incubated during 1984–1985 at ambient hatchery temperatures of 8.3–10.5°C. Although this 60% estimate of blue sac disease was not well quantified, it appears high based on the trend for reduced incidences of blue sac reported from 1977 to 1983 by Simonin et al. (1990) and Symula et al. (1990). Use of chilled water (3.7–7.7°C) by Ostergaard significantly enhanced the survival of these Lake Ontario lake trout and decreased the incidence of blue sac disease, although the exact percent reduction in blue sac disease related mortality was not reported.

Because adult lake trout from Lake Ontario have greater concentrations of TCDD and related aryl hydrocarbon (Ah) receptor agonists than lake trout from any of the other Great Lakes (Zacharewski et al. 1989; DeVault et al. 1989; U.S. EPA 1993), we hypothesized that the TECs in wild Lake

Ontario lake trout eggs would be elevated compared with lake trout eggs from the other Great Lakes. The major route for accumulation of halogenated aromatic chemicals in salmonid eggs is not from contaminated water but rather as translocated lipophilic contaminants, deposited directly from the sexually mature adult females into eggs prior to spawning (Guiney et al. 1979; Niimi 1983; Vodicnik and Peterson 1985). These halogentated aromatic chemicals are then taken up by the developing embryo and sac fry from yolk lipid globules and lipotropic fluid contained inside salmonid eggs (Guiney et al. 1980; Zabel et al. 1995*a*). Recently Walker et al. (1994) reported that maternally derived TCDD resulted in the same signs of toxicity and lethal potency during lake trout sac fry development as exposure of fertilized lake trout eggs to waterborne or injected TCDD.

In this study, background TECs were higher for Lake Ontario eggs than for lake trout eggs obtained from the Crystal Springs hatchery or from Gull Island Shoal, Lake Superior, from 1989 to 1991. Despite the greater TECs in Lake Ontario lake trout eggs in 1991 compared with other Great Lakes, sac fry mortality was uniformly low. The incidence of blue sac disease in the vehicle-exposed lake trout eggs from these two wild Lake Ontario populations (1–11%) is consistent with the finding that total TECs in these eggs were about one third of the TCDD NOAEL for lake trout sac fry mortality for fertilized eggs from Lake Superior (Walker et al. 1991) and Lake Ontario eggs (Table 4). Thus, results of this study show that it is unlikely that lake trout reproduction is currently at risk in Lake Ontario due solely to the effects of background egg TECs on blue sac syndrome-related mortality. Results of a retrospective risk assessment (Cook et al. 1994), however, suggest that, in the period from the mid 1940s to the mid 1970s, the egg TEC exceeded the LD_{100} for causing blue sac syndrome in Lake Ontario lake trout, which would have precluded successful recruitment of lake trout sac fry into the population.

Sensitivity of wild Lake Ontario lake trout to [^{3}H]TCDD-induced early life stage mortality

Our results clearly demonstrate that Lake Ontario lake trout are extremely sensitive and equally responsive when exposed as eggs to waterborne [^{3}H]TCDD as Lake Superior and hatchery derived lake trout sac fry (Table 4). This confirms the strong association that has now been established between TCDD exposure of lake trout eggs via waterborne exposure (Spitsbergen et al. 1991; Walker et al. 1991), injection (Walker et al. 1992), and maternal transfer (Walker et al. 1994) with TCDD-induced blue sac disease. TCDD-induced lesions observed in this study were essentially identical to those induced in the above studies by various routes of exposure.

The failure of wild Lake Ontario lake trout sac fry that are not exposed as eggs to [^{3}H]TCDD to manifest a blue sac disease type of early life stage mortality (Table 3) is because their background egg burdens of TCDD and related chemicals (Table 1) are below the NOAEL for sac fry mortality. When egg concentrations of [^{3}H]TCDD exceed the NOAEL, then dose-related increases in sac fry mortality are seen in Lake Ontario lake trout (Fig. 2), just as is the case in lake trout from Lake Superior and a hatchery source (Fig. 3).

Effect of water temperature on TCDD-induced lake trout early life stage mortality

No significant relationships were established between water temperature and mean egg mortalities or sac fry mortality due to blue sac disease. Eggs from each location, maintained at either 8°C or a 8–3–8°C temperature profile had similar egg mortality and uniformly low sac fry mortality. In addition, for all sources of eggs, exposure to [^{3}H]TCDD resulted in sac fry mortality associated with blue sac syndrome, which was not reduced by lowering the water temperature. The LD_{50} values were all similar, ranging from 42 to 72 pg [^{3}H]TCDD/g egg. Similar results have recently been reported by Fitzsimons et al. (1995) who studied the occurrence of swim-up syndrome in Lake Ontario lake trout and its relationship with rearing temperatures. The incidence of swim-up syndrome (an effect not known to be associated with TCDD exposure in salmonids) was found to be unrelated to incubation temperatures (4 vs. 8°C).

Symula et al. (1990), however, found that reduction in water temperature below 8.3°C for incubation of eyed eggs resulted in a decrease in mortality of sac fry from the same group of Lake Ontario female lake trout in 1982–1984. The reduction in the incidence of mortality associated with blue sac syndrome was from 20% at 8.3°C to 6% at 3.0°C and from 24% at 8.3°C to 9% at 1.7°C. Ostergard (1987) also reported reduced mortality of sac fry when eggs collected in 1984–1985 from Seneca strain lake trout from both Lake Ontario and Seneca Lake were incubated under colder water temperatures (mean 5.3°C as compared with 9.4°C). Water temperatures on shallow lake trout spawning reefs in Lake Ontario fall below 3.0°C in late winter (Casselman 1996), but water temperatures below 3.0°C have not been shown to result in further reduction of sac fry mortality. Symula et al. (1990) reduced water temperatures throughout the incubation period following eying of the eggs, unlike this study in which water temperatures were varied over time (Fig. 1) in a pattern similar to the spawning reef water temperature profile reported by Casselman (1996). The mechanism by which reduced incubation temperature provides partial protection against blue sac disease in some studies but not in others remains to be elucidated. Casselman (1996) associated low sac fry survival on a shallow reef in eastern Lake Ontario with high water tempeatures at the beginning of the egg incubation period. Symula et al. (1990) suggested that temperature may influence blue sac syndrome mortality induced by fungal or bacterial infections. The myoxobacteria *Cytophaga psychrophilia* was isolated from the 1984 lake trout eggs studied by Symula et al. (1990), but the roles of such potential pathogens and possible interactions with chemicals to increase blue sac syndrome mortality remain unknown.

This study was designed to evaluate whether seasonal water temperature fluctuations expected at lake trout spawning reefs in the Great Lakes might affect the sensitivity of lake trout sac fry to TCDD-induced mortality. A constant low temperature effect such as that observed by Symula et al. (1990) could reduce TCDD-induced sac fry mortality. Conversely, it is unknown if the beneficial effect observed by Symula et al. (1990) would occur under the water temperature conditions experienced by lake trout eggs in Lake Ontario. Based on our findings and the water temperature data of Casselman (1996), water temperatures encountered in typical lake trout spawning reefs in Lake Ontario would not be expected to reduce the potency of TCDD to induce lake trout sac fry mortality.

Implications for lake trout reproduction in Lake Ontario

Despite greater TECs (10.5–10.9 pg TCDD equivalent/g egg) in Lake Ontario eggs, lake trout sac fry mortality due to blue sac disease was uniformly low for all control eggs. The LD_{50} and NOAEL values for [^{3}H]TCDD-induced sac fry mortality in Lake Ontario lake trout (Table 4) are very similar to the values Walker et al. (1991) reported for Lake Superior lake trout (65 and 34 pg TCDD/g egg, respectively). Background TECs in Lake Ontario eggs (present at about one third the NOAEL) also did not cause a significant shift to the left of the [^{3}H]TCDD dose–response curve for percent cumulative mortality in comparison with the Lake Superior or hatchery eggs. We believe a fundamental reason for this observation is that the 95% confidence interval for the LD_{50} was generally greater than the background egg TEC. In other words, the bioassay for [^{3}H]TCDD-induced sac fry mortality is not sensitive enough for a background egg TEC of 10.5–10.9 pg/g to cause a statistically significant shift in an LD_{50} that is based on exogenously administered [^{3}H]TCDD. Also, our recent experience indicates that placement of contaminated eggs in an acetone–water exposure medium without chemicals will result in loss of TCDD and related chemicals from the eggs as a [^{3}H]TCDD mass distribution of approximately 1:4 between the eggs and acetone–water is approached. The magnitude of chemical loss from the wild eggs in this study during the 48-h exposure to [^{3}H]TCDD was not measured, so the amount of the background TEC remaining in the eggs for an additive contribution to the observed toxicity is uncertain.

Prince and Cooper (1995) recently reported that killifish (*Fundulus heteroclitus*) embryos collected from a heavily TCDD-impacted estuary of Newark Bay, New Jersey, were resistant to the toxicity of [^{3}H]TCDD when compared with killifish embryos collected from a site reported to be nonimpacted by TCDD. Their study also exposed eggs to [^{3}H]TCDD via acetone–water so Newark Bay contaminants may have been eliminated. More important by comparison to this study, however, is the lack of evidence for a similar difference in the responsiveness of Lake Ontario lake trout and control sac fry to [^{3}H]TCDD exposure of eggs. We conclude that lake trout are unlikely to develop resistance to early life stage toxicity of TCDD and related chemicals under exposure conditions experienced in the Great Lakes.

The slope of the dose response curve for TCDD-induced mortality in Lake Ontario lake trout is steep (Figs. 2 and 3). Previous studies (Walker et al. 1991; Walker and Peterson 1991, 1994) have reported steep slopes for dose–response curves for TCDD and similar compounds in lake trout, rainbow trout, and brook trout (*Salvelinus fontinalis*). Thus, given the statistical power of our assay, strain variability, and the observed steepness in the dose–response curve, TECs in Lake Ontario lake trout eggs from 1991 may be just below a threshold for significant adverse impact. Although PCDD, PCDF, and PCB concentrations appear to be declining in lake trout eggs from the Great Lakes (Cook et al. 1994), a spill or significant non-point-source contamination of TCDD and other related Ah receptor agonists in Lake Ontario could push the current TECs over the critical early life stage mortality threshold for lake trout. In addition, TECs below the threshold for sac fry mortality may cause sublethal effects or enhance nonchemical stressor effects, which may contribute to the lack of lake trout recruitment in Lake Ontario.

In this study, HRGC–HRMS was used to provide sensitive and reliable measurements of PCDDs, PCDFs, and PCBs in egg sample extracts. In addition, we used salmonid-specific TEFs to calculate total TECs present in Lake Ontario lake trout eggs. The TECs present in these eggs in 1991 are below the estimated NOAEL for lake trout sac fry mortality. Lake trout recruitment is probably not currently at risk in Lake Ontario solely because of the effects of TCDD and related chemicals on blue sac syndrome related mortality. The reproductive failure however, of Lake Ontario lake trout in the past, when TCDD and related chemical residues were much higher in eggs, could have occurred because of the bioaccumulation of these chemicals by lake trout and their transfer to the eggs, causing sac fry mortality by a blue sac syndrome.

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Effect of activity level on apparent heat increment in Atlantic cod, *Gadus morhua*

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Abstract: Exercise causes an increase in apparent heat increment in 50–55 cm Atlantic cod, *Gadus morhua*. The duration of apparent heat increment required approximately 51, 83, and 101 h for cod fed a ration equivalent to 2.5% body weight (8°C) and forced to swim at 20, 30, and 40 $cm{\cdot}s^{-1}$ in a Brett-type respirometer. The level of peak apparent heat increment increased by approximately 50, 87, and 116% above routine oxygen consumption rate for fed cod swimming at 20, 30, and 40 $cm{\cdot}s^{-1}$ respectively. The corresponding magnitudes of apparent heat increment increased from approximately 944 to 1910 and 3149 mg $oxygen{\cdot}kg^{-1}$ at 20, 30, and 40 $cm{\cdot}s^{-1}$. These values of oxygen consumption correspond to 8, 16, and 24% of energy ingested over the respective swimming speeds.

Résumé : L'exercice a provoqué une augmentation de l'accroissement apparent de chaleur chez des morues, *Gadus morhua*, de 50 à 55 cm. Lorsque ces poissons ont été soumis à une ration équivalant à 2,5% de leur poids corporel (8°C) puis forcés de nager à 20, 30 ou 40 $cm{\cdot}s^{-1}$ dans un respiromètre de Brett, l'accroissement apparent de chaleur a duré environ 51, 83 et 101 h, respectivement. Pour ces mêmes vitesses de nage, le niveau maximal de l'accroissement apparent de chaleur s'établissait à environ 50, 87 et 116% au-dessus du taux normal de consommation d'oxygène, et la magnitude de cet accroissement était d'environ 944, 1910 et 3149 mg d'$oxygène{\cdot}kg^{-1}$. Ces taux de consommation d'oxygène équivalent respectivement à 8, 16 et 24% de l'énergie ingérée.
[Traduit par la Rédaction]

Introduction

The impact of exercise on digestion has received much attention in the field of human physiology but has remained virtually unexplored in the context of fish biology (Brouns and Becker 1993; Lyndon et al. 1992; Warren 1971). Priede's (1985) review summarizes the general opinion that "in fish ... there must be a continual conflict between the needs of locomotion and SDA [specific dynamic action; the cost of digestion, otherwise known as apparent heat increment (AHI)]. The two metabolic activities are mutually exclusive, and if the fish is swimming at its maximum speed, it presumably cannot proceed with any digestion." This opinion assumes that maximum sustained aerobic metabolism achieved during forced swimming activity is representative of the overall upper limit to aerobic metabolism (Muir and Niimi 1972; Vahl and Davenport 1979). However, if maximum sustained swimming metabolism is not representative of maximum metabolism, and particular organ systems and functions have independent maximum metabolic limits, then both digestion and activity may be able to proceed concurrently without interference.

In the field of fish biology the subject of interaction between activity and digestion is most relevant from a bioenergetic perspective because of the emphasis placed on fish growth and production. The energy balance equation of Winberg (1956) and its derivations (Kerr 1982; Ricker 1971) assumes independence between these two components, swimming activity and the cost of digestion (AHI) (Beamish and Trippel 1990). However, both mammalian literature and recent fish literature support the possibility that activity may impact digestion (Axelsson and Fritsche 1991; Brouns and Becker 1993; Gisolfi et al. 1991; Harris et al. 1991; Moses et al. 1988, 1989; Qamar and Reed 1987). Knowledge of the interaction between components of the energy balance equation, currently thought to be discrete, may in fact yield the key to more accurate models and so better represent the limits experienced by individual fish. Apart from the modelling implications, the potential for interaction determines our understanding of the behaviour and production of both wild fish and their production potential in aquaculture systems.

Apparent heat increment is used here to describe the combined biochemical and mechanical costs of digestion (Beamish 1974; Beamish and Trippel 1990). Our usage supports the NRC's (1981) promotion of common terminology. The term SDA was previously limited to the biochemical cost of digestion.

Our study explores the interaction of swimming activity and AHI by feeding Atlantic cod, *Gadus morhua*, at three different swimming speeds.

Materials and methods

Atlantic cod were captured in the spring of 1993 by trawling off the coast of Halifax, N.S. (Eastern Passage; 44°35′N, 63°30′W). Five cod of similar size (50–55 cm; 1.3–1.5 kg) were maintained in a

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4000-L green fibreglas aquarium continually supplied with filtered seawater (8.0 ± 0.5°C; 33‰ salinity). The aquarium was continually illuminated from above by a single dim light. The cod were fed a mixed ration of previously frozen capelin (*Mallotus villosus*) and squid (*Loligo opalescens*) equivalent to 3.5% body weight (BW) every 2–4 days, between 10:00 and 16:00. Cod were fed on an irregular schedule to eliminate the possibility of cod developing a habitual feeding-related metabolic rhythm.

Apparent heat increment and activity-related metabolism were measured within a 58-L Brett-type respirometer (Brett 1964) modified as described below. A computer automatically opened and closed the respirometer to a flow of fresh seawater on a cycle of 30 minutes and maintained temperature within the respirometer at 8 ± 0.1°C. A feeding tube was mounted at the front of the respirometer to allow feeding the fish within the respirometer.

When the solenoid valve controlling seawater flow was closed the respirometer was completely sealed and the partial pressure of oxygen within the respirometer was progressively reduced by the respiring fish. The partial pressure of oxygen never fell below 70% of saturation. When the valve supplying seawater was open, oxygen partial pressure returned to saturation and the volume of the respirometer was exchanged 4.7 times. This level of flushing prevented accumulation of organic solids such as feces within the respirometer. Mean biological oxygen demand (BOD), measured for at least 5 h before and subsequent to the occupation of the respirometer by each cod, was 9.2 ± 0.7 $\mathrm{mg \cdot h^{-1}}$ for the 58-L volume. Changes in oxygen partial pressure within the respirometer were measured using a Radiometer electrode (E5046).

Water current speed within the respirometer was controlled by adjusting the speed of a vertically mounted impeller. The water-flow profile in the swimming portion of the respirometer was approximately rectilinear with a slightly greater velocity on the upper surface. Water current speed was calibrated to water flow in the centre of the tube.

Each of five fish was weighed and its total length measured before being placed in the respirometer. Cod swam for an initial familiarization period of 72 h at 20 $\mathrm{cm \cdot s^{-1}}$ before being fed a ration of capelin equivalent to 2.5% BW. Cod then swam at 20 $\mathrm{cm \cdot s^{-1}}$ for 6 days, were fed again and swam at 30 $\mathrm{cm \cdot s^{-1}}$ for 6 days, and then were fed a final time and swam at 40 $\mathrm{cm \cdot s^{-1}}$ for 8 days. These swimming durations were based on preliminary observations of differences in duration of heat increment at each swimming speed. The cod were fed by gently lowering their food into the respirometer on a piece of digestible suture. If the cod did not accept the food within 1 h the capelin was removed and feeding reattempted 2 h later. If at this time the cod still did not consume the food the procedure was repeated the following day. As soon as the ration was accepted the respirometer speed was increased to the experimental swimming speed. One cod refused to eat and was force fed by anaesthetizing it with MS 222 and gently forcing the capelin past its oral sphincter using 20-cm forceps. This method was repeated for this fish at all swimming speeds to maintain consistency within its results. This method was presumably more stressful for the fish, and for that reason we postponed measurement of digestive respiration for 5 h postfeeding. Once all three swimming trials were complete the fish was removed from the respirometer, weighed, measured, and returned to the holding tank. Oxygen consumption at each swimming speed was calculated, using the following equation, from the slope of decreasing oxygen tension measured every 10 s for 30 mins of every hour within the completely sealed respirometer:

$$(1) \qquad 0 = \frac{(\Delta P\mathrm{O}_{2\,\mathrm{fish}} - \Delta \mathrm{BOD}) / 156) S (V_{\mathrm{resp.}} - V_{\mathrm{fish}})}{W}$$

where $\Delta P\mathrm{O}_{2\,\mathrm{fish}}$ is the change in torr (1 torr = 133.322 Pa) of oxygen per hour, BOD is biological oxygen demand, *S* is the solubility of oxygen in water at 8°C (9.84 $\mathrm{mg \cdot L^{-1}}$), *V* is respirometer volume in litres, and *W* is the weight of the fish in kilograms.

The typical respiratory response to feeding was a sharp rise in oxygen consumption to a peak followed by a slow decline to a constant nonfed level persistent over at least 72 h. This persistent nonfed level was assumed to be due to exercise alone. The duration of AHI was determined to be complete when 5-h mean oxygen consumption first dropped below the mode of exercise-related oxygen consumption. The mode of exercise-related oxygen consumption was chosen rather than the mean because the occurrence of one or two spikes of oxygen consumption over the 72-h period could considerably bias the mean. Metabolic expenditure due to digestion was calculated for each hour by subtracting the mode of nonfed exercise related respiration from the respiration measured while fish were digesting and swimming. Digestion-related oxygen consumption values were summed over the duration of the feeding related effect to give a measure of the magnitude of AHI. Magnitude, expressed in milligrams $\mathrm{O_2}$ per kilogram was converted to units of energy using an oxycaloric conversion of 14.3 $\mathrm{J \cdot mg^{-1}}$ of oxygen (Brafield 1985; Tandler and Beamish 1979). The level and timing of peak digestion-related oxygen consumption was noted only after the first 3 h of digestion to avoid confusion of AHI with the excitement created by momentarily reducing the water velocity and introducing food into the respirometer.

The effect of swimming speed on the peak, duration, and magnitude of AHI as well as exercise-related oxygen consumption was analyzed for statistical significance using repeated measures analysis of variance (SYSTAT) (Wilkinson 1990).

The maximum swimming speed and associated maximum aerobic swimming respiration of four cod was determined in a separate experiment conducted several months prior to the beginning of feeding experiments. Maximum swimming speed was determined using a "critical swimming protocol" described in detail in Tang et al. (1994). The single exception to this protocol was that water current speed was increased in 10 $\mathrm{cm \cdot s^{-1}}$ increments from 20 $\mathrm{cm \cdot s^{-1}}$ every 30 min until the fish was exhausted.

Maximum oxygen consumption measured using the critical swimming protocol was calculated using eq. 1 and then corrected for fish weight using eq. 2.

$$(2) \qquad \text{Corrected oxygen consumption} = \frac{\text{measured value}}{(\text{body weight})^{0.8}}$$

This value was then compared with maximum oxygen consumption measured during feeding, corrected using eq. 2.

Fresh capelin collected in Newfoundland in July 1993 were sorted and frozen (–15°C) in polyethylene bags within several hours of capture. The capelin were then thawed, decapitated, and used throughout the experiment for regular and experimental feeding. Gravid females were discarded. Capelin are a principal prey of Atlantic cod (Rose and Leggett 1990).

The energy content of 10 dry capelin was determined in an adiabatic bomb calorimeter (Parr Instrument Co., Moline, Ill.) Samples were dried in an 80°C oven for 48 h and then cooled in a desiccator for 24 h before dry weights were determined. Dry weight of capelin represented 22.2 ± 2.5% of their thawed weight. Each dry capelin was homogenized using a mortar and pestle. Two 1-g samples of one capelin were burned in the bomb calorimeter to determine the variability within a sample. The 10 homogenized capelin were then combined. Three of 10 subsamples (1 g each) were chosen for burning using a random number table. The mean energy content of the capelin was 4.9 $\mathrm{kJ \cdot g^{-1}}$ fresh weight. A 40-cm piece of absorbable suture, the length consumed by cod with each meal, was also burned in the bomb calorimeter and its energy content determined to be insignificant (0.36 kJ).

Nitric acid liberated as the byproduct of combustion was collected from each of the five samples and used to determine the nitrogen content of the capelin. The nitric acid was rinsed from the bomb

Table 1. Apparent heat increment for five cod fed a ration equivalent to 2.5% body weight and forced to swim at 20, 30, and 40 $\mathrm{cm \cdot s^{-1}}$ (values do not include the cost of swimming).

Fish	Speed ($\mathrm{cm \cdot s^{-1}}$)	Nonfed swimming O_2 consumption ($\mathrm{mg \cdot kg^{-1} \cdot h^{-1}}$)	Apparent heat increment: Duration (h)	Apparent heat increment: Peak ($\mathrm{mg \cdot kg^{-1} \cdot h^{-1}}$)	Apparent heat increment: Magnitude ($\mathrm{mg \cdot kg^{-1}}$)
F1 (1.53 kg, 55 cm)	20	91	38	30.8	846.3
	30	109	74	66.5	2302.0
	40	109	81	118.1	3031.5
	60*	173*			
F2 (1.53 kg, 55 cm)	20	83	49	31.1	756.1
	30	81	99	59.1	1984.1
	40	71	118	103.7	2894.2
	60*	236*			
F3 (1.53 kg, 55 cm)	20	84	66	33.4	830.6
	30	85	88	38.2	1211.7
	40	92	121	61.6	3186.0
	60*	98*			
F4 (1.53 kg, 55 cm)	20	65	60	35.5	1014.3
	30	63	70	56.6	2005.8
	40	65	74	98.7	3059.3
	60*	113*			
F5 (1.53 kg, 55 cm)	20	82	43	55.1	1274.1
	30	134	80	67.9	2046.1
	40	133	110	65.3	3573.9

Note: Modal nonfed swimming oxygen consumption at each swimming speed is reported separately. Values with asterisks are maximum forced swimming speeds and associated oxygen consumptions for four fish (measured several months prior to feeding experiments and corrected for a 1 kg fish). Fish F4 was force fed at each swimming speed.

chamber with distilled water and pipetted into glass vials. The samples were then titrated with 0.001 M NaOH using a phenolthalein indicator (pH 7–8). The capelin contained a mean nitrogen content of 3.4 ± 0.3% fresh weight. These values, converted to protein using a multiple of 6.25 (Jobling and Davies 1980), equal 206 ± 17 mg protein per gram wet weight of capelin. This value is in the range cited for a variety of diets (Jobling and Davies 1980; Lie et al. 1988; Lyndon et al. 1992).

Results

Three components characterize the cod's metabolic response to feeding: the duration of increased oxygen consumption, the peak level of this increase, and the magnitude of the response (AHI) (Table 1, Fig. 1). Each of these terms describes only oxygen consumption in excess of that required for swimming at each of the three swimming speeds. For each fish the duration of AHI increased significantly with swimming speed ($P < 0.001$), requiring 51.2 ± 9.4 (mean ± SD), 82.7 ± 9.0, and 100.8 ± 18.6 h at 20, 30, and 40 $\mathrm{cm \cdot s^{-1}}$, respectively (Fig. 2A).

Peak oxygen consumption increased significantly with swimming speed ($P < 0.001$) (Fig. 2B). Digestion-related peak oxygen consumption reached 37.2 ± 7.2, 57.7 ± 8.2, and 89.5 ± 20.8 $\mathrm{mg \cdot kg^{-1} \cdot h^{-1}}$ above swimming oxygen consumption at 20, 30, and 40 $\mathrm{cm \cdot s^{-1}}$, respectively. These values represent an increase of 47 ± 12, 72 ± 11, and 112 ± 28% above routine (20 $\mathrm{cm \cdot s^{-1}}$) nonfed oxygen consumption rate. The time to reach peak oxygen consumption did not differ appreciably with swimming speed and persisted for 1–2 h. Peak oxygen consumption generally occurred within the first 20 h following feeding.

The magnitude of AHI increased significantly with swimming speed ($P < 0.001$) (Fig. 2C). AHI required 944 ± 160, 1910 ± 279, and 3149 ± 185 mg $\mathrm{O_2 \cdot kg^{-1}}$ at 20, 30, and 40 $\mathrm{cm \cdot s^{-1}}$, respectively. Using an oxycaloric conversion of 14.3 $\mathrm{J \cdot mg^{-1}}$ oxygen these values equal 13.5 ± 2.3, 27.3 ± 4.0, and 45.0 ± 2.6 kJ of energy consumed for AHI while swimming at 20, 30, and 40 $\mathrm{cm \cdot s^{-1}}$. As a proportion of gross energy intake they represent 8, 16, and 26% of ingested energy at 20, 30, and 40 $\mathrm{cm \cdot s^{-1}}$.

Active oxygen consumption did not show a consistent pattern of increase over the swimming speeds used ($P = 0.381$). Maximum oxygen consumption measured using the critical swimming protocol is listed in Table 1 for four of the five fish. Maximum swimming speed attained for 30 min using this protocol was 60 $\mathrm{cm \cdot s^{-1}}$ for each fish.

Fig. 1. Patterns of apparent heat increment for each fish at 20 (open), 30 (hatched), and 40 $\mathrm{cm \cdot s^{-1}}$ (solid). Values represent running averages of five, 1-h oxygen consumption rates. Oxygen consumption was extrapolated for the final hours for fish F3 at 40 $\mathrm{cm \cdot s^{-1}}$ because of equipment failure. Axes differ for each panel.

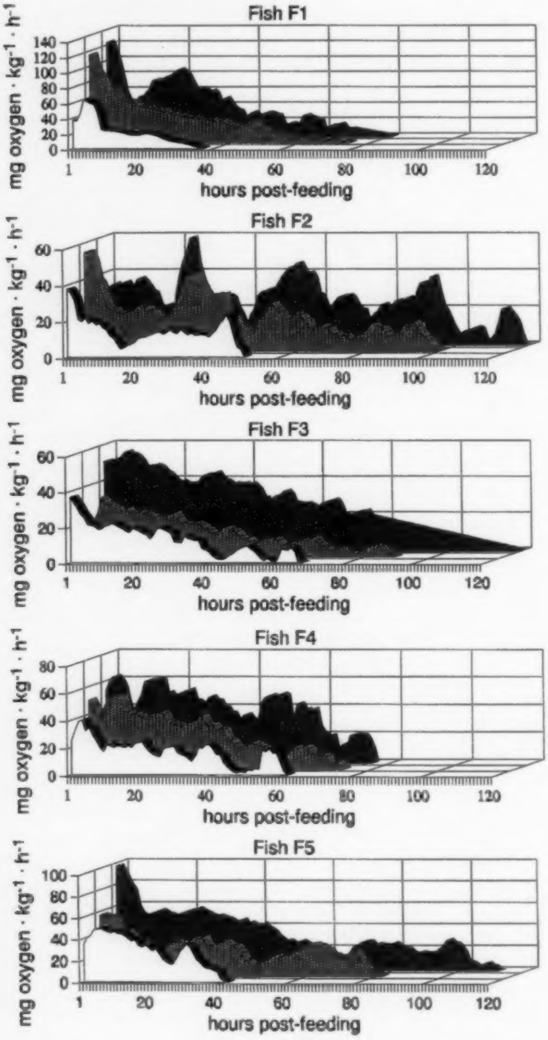
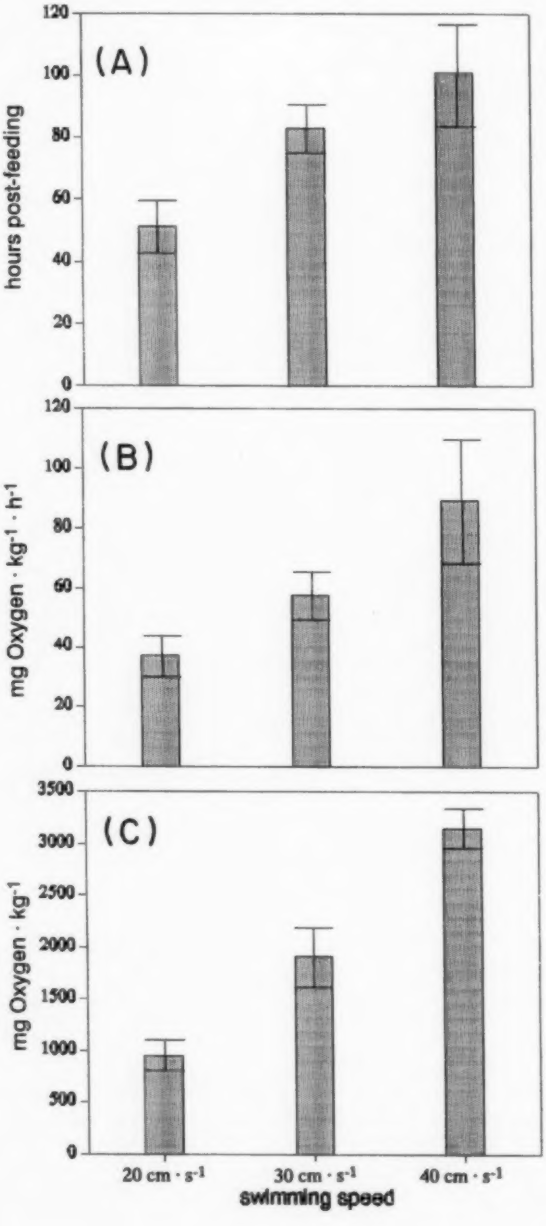


Fig. 2. (A) Mean duration (hours post-feeding), (B) peak (mg $\mathrm{O_2 \cdot kg^{-1} \cdot h^{-1}}$), and (C) magnitude (mg $\mathrm{O_2 \cdot kg^{-1}}$) of apparent heat increment (± SD) for cod fed a ration equivalent to 2.5% body weight and swimming at 20, 30, and 40 $\mathrm{cm \cdot s^{-1}}$.



Discussion

Changes in swimming speed significantly affected the peak, duration, and magnitude of AHI in Atlantic cod (50–55 cm). Such an interaction has been postulated by numerous investigators including Warren (1971), Beamish (1974), and Priede (1985). Beamish (1974), however, did not detect an effect of swimming speed on AHI when juvenile largemouth bass, *Micropterus salmoides*, swam at three moderate swimming speeds.

Peak levels of oxygen consumption increased by approximately 47–112% of routine (20 $\mathrm{cm \cdot s^{-1}}$) nonfed values when swimming speed was increased from routine to 66% of maximum sustainable swimming speed (40 $\mathrm{cm \cdot s^{-1}}$). The cost of digestion per unit time, as indicated by increased peak oxygen consumption, is greater for the actively swimming fish. As will be described below this increased cost per unit of time may be related to either increased absorption of nutrients aided by increased cardiac output or substrate switching from lower cost fats and carbohydrates to proteins (Clausen and Trap-Jensen 1970; Harris et al. 1991). Peak AHI is the highest oxygen consumption achieved by the fish during digestion.

When peak oxygen-consumption values are expressed as multiples of routine oxygen consumption they range from 1.49 times routine at 20 $cm \cdot s^{-1}$ to 2.15 times routine at 40 $cm \cdot s^{-1}$. These values are very similar to those observed for other species over a range of experimental treatments (Tandler and Beamish 1979; Jobling and Davies 1980; Muir and Niimi 1972; Vahl and Davenport 1979). Saunders (1963) reported peak oxygen consumption as 1.6 times routine values for Atlantic cod fed an indeterminate amount of herring in a group respirometer. Saunders' (1963) oxygen consumption values for routine activity and peak AHI were 72 and 113 $mg \cdot g^{-1}$, respectively, at 10°C (cod size ranged from 0.84 to 1.27 kg). Absolute values for unfed routine and peak fed oxygen consumption in our study were 80.0 and 118.8 $mg \cdot kg^{-1} \cdot h^{-1}$, respectively.

When peak oxygen consumption values are expressed as absolute oxygen consumption (peak AHI + swimming oxygen consumption) they represent 88 ± 24, 106 ± 21, and 136 ± 29% of metabolic scope for activity, when fish swim at 20, 30, and 40 $cm \cdot s^{-1}$, respectively. These figures are based on maximum oxygen consumptions measured using the critical swimming protocol. Similar results are achieved by using maximum swimming oxygen consumption values observed by Nelson et al. (1994) and Y. Tang, J.A. Nelson, S.P. Reidy, S.R. Kerr, and R.G. Boutilier (Department of Biology, Dalhousie University, Halifax, NS B3H 4J1, Canada, unpublished data) for similar-size Atlantic cod. Therefore, it appears that the cod must have a metabolic scope for digestion that is independent of and higher than the metabolic scope for activity, as normally defined. That the resting, unfed cod devotes 40% of its cardiac output to the gastrointestine also indicates that, for cod, digestion may be a priority (Axelsson and Fritsche 1991). Priede (1985) suggests that the occurrence of a higher scope for digestion is evolutionarily related to the pressure on relatively inactive fish to devote resources to digesting less frequent but larger meals.

Like peak AHI, the hourly costs of digestion increased over the entire time course of AHI as swimming speed increased. A possible explanation for the increased costs of digestion at higher swimming speeds may be linked to substrate switching from fat and carbohydrate to protein. It is possible that, at lower swimming speeds, the cod had a positive energy balance and were able to store this excess energy. At the higher swimming speeds a negative energy balance may have developed if the demands of exercise and digestion exceeded the energy gained from the ration. As the cod's stores of fat and carbohydrate were used up it may have had to mobilize energy stored as protein. The deamination and conversion of protein to fat and carbohydrate is energetically costly (Beamish and Trippel 1990). In fact, the commonly observed disproportionate increase in AHI with increasing amounts of protein in the diet is thought to be due to the high cost of deaminating excess amino acids (Beamish and Trippel 1990).

The duration of AHI increased with activity level, requiring 51, 83, and 101 h at 20, 30, and 40 $cm \cdot s^{-1}$, respectively. Gastric evacuation rate and the duration of associated increases in metabolism are linearly related, and both are altered by variables such as temperature, ration size, and fish weight (Jobling and Davies 1980; dos Santos and Jobling 1991a, 1991b; Tandler and Beamish 1979). The increased duration of AHI with activity level may promote more complete digestion and absorption of nutrients by decreasing gastric evacuation rate. As gastric evacuation rate is decreased, the contact time for the digested ration with the epithelial cells lining the intestine is increased. Increased absorption of nutrients may be partly responsible for the increased cost of digestion with increasing swimming speed.

The total amount of oxygen consumed by the fish for the process of digestion is referred to as the magnitude of AHI. This magnitude is a function of both the peak and duration of oxygen consumption and represents the total cost of digesting a particular ration (Jobling 1981). Thus, the increased cost of digestion with swimming speed results from both an increased duration of AHI and increased momentary costs of digestion. Increases in ration size have previously been shown to cause a similar increase in the magnitude of AHI in fish (Jobling and Davies 1980; Tandler and Beamish 1979; Vahl and Davenport 1979). Tandler and Beamish (1979) observed that, as meal size increased, biochemical heat increment increased exponentially whereas mechanical heat increment increased curvilinearly. This biochemical elasticity may reflect increased costs of active transport in the gut as well as increased conversion and storage of absorbed nutrients.

The proportion of energy ingested that must be expended during digestion is referred to as the coefficient of AHI. In our study the coefficient increased with activity level, requiring approximately 8, 16, and 26% of energy ingested at swimming speeds of 20, 30, and 40 $cm \cdot s^{-1}$, respectively. For other fish, previously observed values for the coefficient of AHI obtained at routine or low swimming activity levels generally varied from 7.5 to 24% of energy ingested (Beamish 1974; Jobling and Davies 1980; LeGrow and Beamish 1986; Muir and Niimi 1972; Vahl and Davenport 1979). Beamish and Trippel (1990) report values of AHI ranging from 3% to a considerable 41% for fish fed natural rations and swimming at very low speeds. Therefore, the cost of digestion can significantly reduce the amount of energy available for growth and other processes that occur between meals.

The increased cost of digestion with increases in activity level may be related to greater transport of nutrients from the intestine or increased oxygen consumption by epithelial carrier systems. Both of these may be accomplished through increased cardiac output and possibly increased perfusion of the gut. Clausen and Trap-Jensen (1970) observed an increase in blood flow to the gastrointestine resulting from increased cardiac output during activity in humans. Axelsson and Nilsson (1986) observed a 1.46-fold increase in cardiac output in 37–41 cm cod exercised at approximately 26 $cm \cdot s^{-1}$ for 15 min.

Axelsson and Fritsche (1991) observed that feeding increased coeliac and mesenteric blood flow by 72 and 42%, respectively, in resting Atlantic cod (38–45 cm) following a meal of between 2.5 and 3.5% BW. In contrast, exercise caused an approximately 32% diversion of blood flow from the coeliac and mesenteric arteries during 10 min of exercise at 30 $cm \cdot s^{-1}$ (Axelsson and Fritsche 1991). It is difficult to surmise from these data alone what the combined blood flow would be in an active fed fish exercised over a period of days. It is worth noting, however, that in terms of blood flow, the gut of a fed cod is a very important organ.

Given the importance of the effect of swimming speed on the magnitude of AHI it seems, at first glance, surprising that unfed swimming costs did not show a clear pattern of increase with increased swimming speed. The processes of digestion and locomotion are, however, indirectly linked and each may be subject to its own limitations and sensitivities. The patterns and values of active oxygen consumption noted in our study were also observed by Tang et al. (1994) and Nelson et al. (1994) for Atlantic cod of similar size over the same range of swimming speeds. The similarity in values of oxygen consumption at each swimming speed also confirms our assumption that the sequence of swimming speeds used in our study (i.e., increasing vs. randomized) did not affect swimming performance.

For Atlantic cod the energetic efficiency of digestion was reduced as activity level increased. The range of swimming speeds investigated is well within the range of speeds (0.5–1.0 body $lengths \cdot s^{-1}$) determined using acoustics for migrating cod in the wild (G.A. Rose, Department Fisheries and Oceans Canada, P.O. Box 5667, St. John's, Nfld., personal communcation). This interaction between activity and the cost of digestion is important as these two components of fish bioenergetic models, activity and AHI, have conventionally been assumed to be independent. The conjecture by Priede (1985) that activity and digestion must be "mutually exclusive" is incorrect when applied to cod. Cod seem to be capable of absorbing and distributing enough oxygen to both their muscle and gastrointestine to fuel the energetic demands of each.

Future study of the interaction of activity on AHI should be focussed on the physiological impacts of activity on gastrointestinal efficiency. As in humans, exercise appears to alter intestinal blood flow, nutrient absorption, and gut transit time in cod and perhaps other fish. Measures of the impact of changing swimming speed on each of these variables as well as cardiac output in fed and unfed fish would help to confirm the possible mechanisms of action proposed in this paper.

Bioenergetic models should incorporate the observed energetic impact of activity level on digestion. In addition, models may benefit from the understanding that, in cod, metabolic scope for activity, as traditionally measured by swimming performance, is not wholly representative of total metabolic scope. Maximum oxygen consumption appears to be associated with a combination of activity metabolism and AHI.

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Matriarchal population structure of introduced rainbow trout (*Oncorhynchus mykiss*) in the Lake Ontario watershed

Lucy A. Dueck and Roy G. Danzmann

Abstract: A survey of 761 rainbow trout (*Oncorhynchus mykiss*) from eight naturalized populations in Lake Ontario revealed that significant differences in genetic architecture have developed among three groups at the lakewide geographic level. Based on maternal lineage determined by restriction enzyme analysis of mitochondrial DNA, northern (Ontario) populations segregated from both migratory and isolated eastern (New York) populations, which also differed from each other regionally. New York populations were distinguished by lower diversity and by the predominance of one haplotype in fish from migration-accessible streams. Ontario populations had higher, more evenly distributed diversity for both migratory and isolated populations. Haplotype frequency distribution for one Ontario population was dissimilar from that in neighbouring streams colonized earlier. A potential for differentiation was found between local sites within two Ontario streams. Repeated, sporadic introductions from multiple sources and population expansion probably account for high haplotypic diversity in Ontario populations. The lower diversity and distinctive haplotype of New York migratory populations, however, may be a consequence of recent massive stocking, which likely altered the original trajectory of population structuring.

Résumé : L'examen de 761 truites arc-en-ciel (*Oncorhynchus mykiss*) provenant de huit populations établies dans le lac Ontario a mis en évidence des différences significatives, sur le plan de la structure génétique, qui sont apparues entre trois groupes à l'échelle du lac. La détermination des lignées maternelles par analyses de l'ADN mitochondrial au moyen d'enzymes de restriction montre que les populations du nord (Ontario) se sont détachées de la population migratrice et de la population isolée de l'est (État de New York), qui diffèrent aussi l'une de l'autre sur le plan régional. Les populations de l'État de New York se caractérisent par une moins grande diversité et par la prédominance d'un haplotype chez les poissons provenant des cours d'eau accessibles aux migrateurs. Les populations de l'Ontario, tant les migratoires que les populations isolées, sont plus uniformément et davantage diversifiées. La distribution en fréquence des haplotypes d'une de ces populations diffère de celle de truites provenant de cours d'eau voisins, mais colonisés plus tôt. Les auteurs ont trouvé qu'il existe un potentiel de différentiation entre des endroits précis dans deux cours d'eau de l'Ontario. L'introduction sporadique et répétée de truites provenant de sources multiples et l'accroissement des populations expliquent probablement l'importante diversité des haplotypes dans les populations de l'Ontario. La diversité réduite et l'haplotype caractéristique des populations migratoires de l'État de New York peuvent cependant résulter des récentes opérations d'ensemencement massif qui a sans doute modifié l'évolution originelle de la structure des populations.
[Traduit par la Rédaction]

Introduction

Knowledge of genetic population structure is essential for effective stewardship of fishery resources to help maximize diversity, delineate distinct conservation units, and tailor specific management strategies (Allendorf et al. 1987; Chakraborty and Leimer 1987; O'Brien 1994). Through the interaction of processes over an evolutionary time scale, endemic species are usually expected to have some population structure (Avise 1994). This structure can often be recognized as a geographic patterning of the genetic diversity, reflecting both historical colonization events and divergence due to adaptation to local conditions (Bermingham and Avise 1986; Billington and Hebert 1991; Carvalho and Hauser 1994). Population subdivision and localized adaptations may be further enhanced by behavioural mechanisms in salmonids that display natal site imprinting and homing, with consequent reproductive isolation (Horrall 1981; Altukhov and Salmenkova 1987; Grewe and Hebert 1988; Carvalho and Hauser 1994).

Introduced species, on the other hand, are often a random collection of genotypes with widely varying physiological, behavioural, and life-history responses to environmental variables. Since they have no pre-existing history or affinity to their new local conditions, population structure cannot be expected initially (Altukhov and Salmenkova 1987), but they may undergo rapid selection from the alien pressures of their new habitat (Gharrett and Thomason 1987). Introduced salmonids that establish self-sustaining populations are likely to develop new localized adaptations and genetic structure

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even more quickly, depending on the degree of isolation experienced. If this structure is now recognizable, it could prove meaningful for effective management.

From their native range in Pacific drainages, rainbow trout (*Oncorhynchus mykiss*) have become the most widely distributed salmonid in the world, owing to more than a century of introductions (MacCrimmon 1971). The species' phenotypic plasticity and high genetic diversity likely account for its phenomenal success at colonization (Hershberger 1992), suggesting that rainbow trout is able to rapidly adapt to local environments. In the Great Lakes region, rainbow trout are now an integral component of the biotic community, and contribute significantly to an economically important sport fishery for salmonids (Talhelm 1988; Krueger and May 1991). However, recent concerns about imbalanced food webs, continued stocking, and declining natural reproduction (Jones et al. 1993; Rand et al. 1993; Ontario Ministry of Natural Resources, unpublished data), make it imperative to gain a better understanding of the genetic structure for naturalized rainbow trout. If populations have become segregated among drainages and potentially adapted locally over the past 100 years, knowledge of that structure would be invaluable in addressing these concerns.

Rainbow trout were first introduced into the Lake Ontario watershed in 1878 when first-generation progeny of native West Coast fish were released from a Caledonia, N.Y., hatchery (Green 1880; MacCrimmon 1971; MacCrimmon and Gots 1972). Their broodstock source is generally considered to be the McCloud River in north-central California, home to both resident and anadromous (called steelhead) life-history variants (Green 1880; Wales 1939; Dollar and Katz 1964; MacCrimmon 1971; Gall and Crandell 1992). About 20 years later, steelhead from northern coastal California were also planted (Ravenel 1897; Shebley 1922; MacCrimmon and Gots 1972). By the turn of the century, 409 000 rainbow trout had been introduced into New York waters of Lake Ontario (MacCrimmon and Gots 1972), initiating the potential for early population development in the watershed. By the late 1920s, successful reproduction was reported in both the Finger Lakes area and the Salmon River near the eastern basin (MacCrimmon and Gots 1972). At that time, Ontario began developing its Normandale rainbow trout strain for later introduction into north-shore tributaries (MacCrimmon and Gots 1972; Christie 1973). Stocking from multiple sources has continued and escalated since then to a total of 14.6 million rainbow trout from 1968 through 1993, with two-and-a-half times more stocking done by New York than by Ontario agencies. In many Lake Ontario tributaries, self-sustaining populations eventually developed that had a migratory life history analogous to that of coastal steelhead. Since migratory rainbow trout in the Great Lakes also home to their natal stream to spawn (Scheer 1939; Dodge 1972; Ricker 1972; Horrall 1981; Quinn 1990), it is possible that genetically distinct, semi-isolated subpopulations have developed in the Lake Ontario watershed.

Although many factors could have influenced population development in the new freshwater ecosystem (Carvalho 1993), evaluation of genetic responses by rainbow trout to Great Lakes introduction is sparse. Biette et al. (1981) reported differing migration times of fish among spawning streams, indicating the potential formation of discrete stocks. Genetic studies using allozyme data have produced conflicting evidence on differentiation among naturalized Great Lakes populations. In the Lake Superior watershed, Krueger and May (1987) and Krueger et al. (1994) found significant genetic differences associated with geographic distance among streams but not between migration times. Ferguson and Ihssen (1991) also detected significant heterogeneity among populations in a wider multilake survey but found no evidence for geographic patterning. A confounding factor often noted using allozyme evidence, however, is the significant variation in allele frequencies among year-classes (Reisenbichler et al. 1992).

The objective of our study was to determine if a geographic pattern to genetic structuring has developed for naturalized rainbow trout populations from the Lake Ontario watershed, using restriction fragment length polymorphism (RFLP) analysis of the whole mitochondrial DNA (mtDNA) molecule as the survey tool. This type of analysis provides an intrinsic marker of maternal lineage for each fish. Population studies using mtDNA markers have proved to be excellent for quantifying maternal-based genetic structure (Avise et al. 1979; Avise 1986; Ferris and Berg 1987; Moritz et al. 1987; Hallerman and Beckmann 1988; Wirgin and Waldman 1994). Initial investigations using mtDNA RFLPs on naturalized rainbow trout from one Lake Ontario drainage have disclosed extensive genetic variability (Danzmann et al. 1993; Ferguson et al. 1993). However, the technique has not been widely employed to ascertain the extent of population segregation that has developed in a naturalized species or to track stocking influence as suggested by Billington and Hebert (1991). Our findings about this population structure have immediate practical application in management decision-making for Lake Ontario. The genetic profiles of naturalized populations can then be compared with those of stocking strains to determine the survival, homing, and reproductive success of different strains. It also provides baseline data from which to monitor changes in genetic architecture and to assess the effect of continued stocking on established populations where the loss of diversity in a dynamic habitat would be inadvisable.

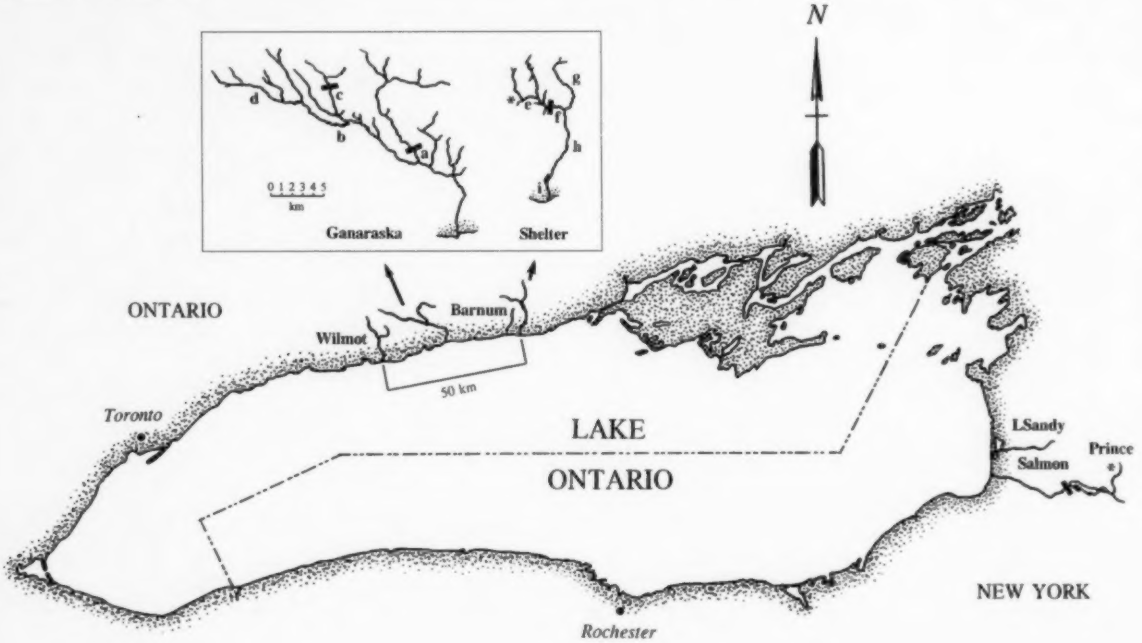
Methods

Sampling

A total of 761 fish were sampled during 1992–1993 from Lake Ontario drainages where self-sustaining populations occur (Fig. 1). These streams included four tributaries where stocking was discontinued about 20 years ago on the north-central shore in the province of Ontario (Wilmot Creek, Ganaraska River, Barnum House Creek, Shelter Valley Brook) and two tributaries on the east shore in New York state (Little Sandy Creek, Salmon River). All six drainages are accessible to migration from Lake Ontario. In addition, fish were sampled in sections of two streams that are isolated by dams from lake migration: upper west Shelter Valley Brook in Ontario and upper Salmon River (Prince Brook) in New York.

Juvenile rainbow trout ($n = 301$) were collected by various methods (angling, electrofishing, dipnetting, and rearing) from several sites within the Ganaraska and Shelter drainages (Fig. 1, inset) for hierarchical analysis of population structure at a local level. In addition, juveniles were collected from Wilmot Creek ($n = 68$) by Ontario Ministry of Natural Resources (OMNR) and from the three New York steams ($n = 65$) by New York Department of Environmental Conservation (NYDEC) for regional and lakewide levels of hierarchical analysis. Two sites (Trout and Orwell brooks)

Fig. 1. Study areas in the Lake Ontario watershed. The four north-shore tributaries in Ontario (Wilmot Creek, Ganaraska River, Barnum House Creek, Shelter Valley Brook) and two east-shore tributaries in New York (Little Sandy Creek, Salmon River) are accessible to lake migration, except two isolated sections (denoted by asterisks) of Shelter and Prince Brook (Salmon River headwater). Heavy bars indicate dams, which are barriers to gene flow. Study sites at the local level of hierarchical analysis for Ontario streams are shown in the inset (four on the Ganaraska River. (*a*) Canton, (*b*) Osaca, (*c*) Elizabethville, and (*d*) Kendal; five on Shelter Valley Brook: (*e*) Isolated, (*f*) West, (*g*) East, (*h*) Main, and (*i*) Lower).



were sampled within the lower Salmon River but combined because of low sample size. Adult fish ($n = 297$) were sampled from anglers' creels at the Ontario study streams during spring and fall runs. Samples were also taken from 22 adult fish collected at the Ganaraska River by OMNR personnel for other purposes. Tissues for mtDNA analyses (liver, heart, and muscle in juveniles; liver only or eggs in adults) were taken from all fish, preserved in sterile TEK buffer (Chapman and Powers 1984) modified with 8.5% sucrose, transported on ice to the University of Guelph, and processed within 1–4 days of sampling.

Laboratory procedures

Mitochondrial DNA was isolated from fresh tissue using methods modified from Chapman and Powers (1984). Total DNA (mitochondrial and nuclear) was extracted from frozen tissue using the protocol of Taggart et al. (1992). DNA was digested with up to 15 hexameric restriction enzymes (*Apa*I, *Ava*I, *Bam*HI, *Bgl*II, *Csp*45I, *Dra*II, *Eco*RV, *Hinc*II, *Hind*III, *Hpa*I, *Mam*I, *Nhe*I, *Sna*BI, *Ssp*I, *Sty*I) found to be polymorphic in rainbow trout from eastern North America (Danzmann et al. 1993; Ferguson et al. 1993). Fragments were separated on 0.8–1.2% agarose gels and visualized either by ethidium bromide staining or by chemiluminescent detection on lumigraphs using the Boehringer-Mannheim AMPPD-DIG system described by Höltke et al. (1992). Prior to detection, gel fragments were transferred to Hybond N (Amersham Corp.) using a Pharmacia LKB vacublotter according to manufacturer's specifications, except that gels were not depurinated.

Data analysis

Visualized fragments were scored for "phenotype" (A, B, C, D, or E banding pattern) of each restriction enzyme used. Most phenotypes (39) have been established for the enzymes used (Danzmann et al. 1993; Ferguson et al. 1993). For those enzymes demonstrating new phenotypes in this study, a Summa Sketch II Plus digitizer tablet was used with a GWBasic program CUTSITES to quantify fragment lengths in relation to lambda standards (Southern 1979; Schaffer and Sederoff 1981). Unique combination of phenotypes for the 15 polymorphic restriction enzymes were used to designate mtDNA haplotypes (clones) with a GWBasic program CLONER. All GWBasic programs were written by D. Dueck (unpublished).

Since there is a significant direct relationship between number of haplotypes found and number of individuals sampled (Grewe and Hebert 1988), effectiveness of different sample sizes for adequately estimating genetic variation in these populations was tested. A GWBasic program SAMPDIST was used to generate a distribution of haplotype accumulation by randomly selecting samples from the data set, and to calculate the log-curve equation by a least-squares fit. Fifty randomizations were performed for each sampling distribution generated and the average simulation values plotted along with actual haplotype accumulation. This technique is similar to Hebert et al.'s (1987) approach, which suggests sampling adequacy when an asymptote is reached. Without a priori knowledge of the diversity present, a minimum sample size of 60 individuals has been recommended for 95% confidence in detecting haplotypes at frequencies of 5% or above (Grewe et al. 1993).

Two measures were employed to quantify genetic variation. The probability of selecting two individuals with different haplotypes from the same group was calculated using nucleon diversity (h) estimates according to Nei and Tajima (1981). Partitioning of genetic variation at different hierarchical levels was done using frequencies of equidistant haplotypes in the AMOVA program of Excoffier et al. (1992). AMOVA also tests the significance of variance components and Φ-statistics (F_{ST} analogs) by a permutational approach, thus eliminating the normality assumption for an analysis of variance.

Heterogeneity testing of haplotype distributions among collection groups in three hierarchical levels was performed with a Monte Carlo simulation of the χ^2 distribution (Roff and Bentzen 1989), using the MONTE program from McElroy et al.'s (1992) Restriction Enzyme Analysis Package (REAP). One thousand replications were run for all tests, and if $P \leq 0.10$, most were retested with 5000 replications. Results are presented as sets of pairwise comparisons, with an overall probability value also calculated for all samples together within each table. A significance level of $\alpha = 0.05$ was set a priori, but when multiple tests were made simultaneously (i.e., pairwise comparisons in a table), adjustments to significance level calculated by the sequential Bonferroni method (Cooper 1968; Holm 1979; Rice 1989) are provided.

Relationships among populations are illustrated by a genetic similarity phenogram, which is based on haplotype frequency differences and determined by Manhattan metric distances (D_M) between populations, with values ranging from 0 to 1 (Sneath and Sokal 1973; Nei 1987). D_M was then used in an unweighted-pair-group method of average linkages (UPGMA) algorithm to generate branching points and clustering distances among populations, with branch lengths equal to $0.5D_M$ (Nei 1987). Both calculations were performed in a GWBasic program MMUPGMA.

Evolutionary divergence among haplotypes was not incorporated into data analyses for this study, since multiple introductions from different source populations are expected to uncouple any phylogeographic patterning that had developed in a totally different environment prior to transplantation. Therefore, the only meaningful information on population structure for an introduced species will come from haplotype frequencies as a consequence of genetic drift, migration, and founder effects (P. Bentzen, School of Fisheries, University of Washington, Seattle, Wash., personal communication). In this study, haplotypes were weighted equally in analyses that addressed potential structuring among populations.

Results

Genetic variation

Twenty-two haplotypes[2] were detected among the 761 rainbow trout collected from Lake Ontario drainages. Thirteen haplotypes were found (clones 1, 2, 3, 4, 6, 8, 9, 10, 13, 18, 21, 24, 28) of the 30 previously identified by Danzmann et al. (1993). In addition, nine new haplotypes (clones 31, 32, 34, 36, 37, 38, 39, 40, 41) were discovered among 50 of the 761 individuals (Table 1). Five of these new haplotypes (clones 32, 34, 36, 37, 41) were the result of different combinations of existing phenotypes. The other four resulted from different fragment banding patters (new phenotypes) for a restriction enzyme: clone 31, *Nhe*I-C; clone 38, *Bam*HI-C; clone 39, *Dra*II-D; and clone 40, *Ava*I-E. The phylogenetic positions of these 9 new haplotypes relative to the 30 previously described by Danzmann et al. (1993) are illustrated on a Wagner parsimony network (Fig. 2).

A maximum of 140 restriction sites were surveyed in this study, representing approximately 5% of the mitochondrial

Table 1. Unique combinations of banding patterns that designate new haplotypes found in Lake Ontario rainbow trout.

Clone	Restriction enzyme														
	*Ava*I	*Apa*I	*Bam*HI	*Bgl*II	*Csp*45I	*Dra*II	*Eco*RV	*Hinc*II	*Hind*III	*Hpa*I	*Mam*I	*Nhe*I	*Sna*BI	*Ssp*I	*Sty*I
31	B	—	A	C	C	A	—	B	—	—	B	C*	A	A	—
32	—	—	A	B	C	B	A	B	—	A	B	B	B	A	—
34	D	—	A	A	B	A	A	B	—	B	A	A	A	A	—
36	D	—	—	A	A	A	—	—	—	—	B	—	A	—	—
37	A	—	A	A	A	A	A	B	A	A	B	A	A	A	—
38	A	A	C*	A	C	A	A	B	A	A	B	A	A	A	A
39	D	—	A	C	A	D*	A	A	A	A	B	A	A	A	—
40	E*	—	A	A	A	A	A	B	A	A	B	A	A	A	—
41	C	—	A	A	B	A	—	B	—	—	A	A	A	B	—

Note: Clones 1–30 were previously designated by Danzmann et al. (1993), and clones 33 and 35 are described by Danzmann et al. (1994).

*Fragment lengths (kbp) for new banding patterns: *Nhe*-C, 9.5, 7.23; *Bam*HI-C, 8.45, 4.34, 4.21; *Dra*II-D, 5.8, 2.57, 2.4, 1.9, several <0.60; *Ava*I-E, 6.42, 2.24, 1.8, 1.5(2?), 1.28, 1.24, 0.61.

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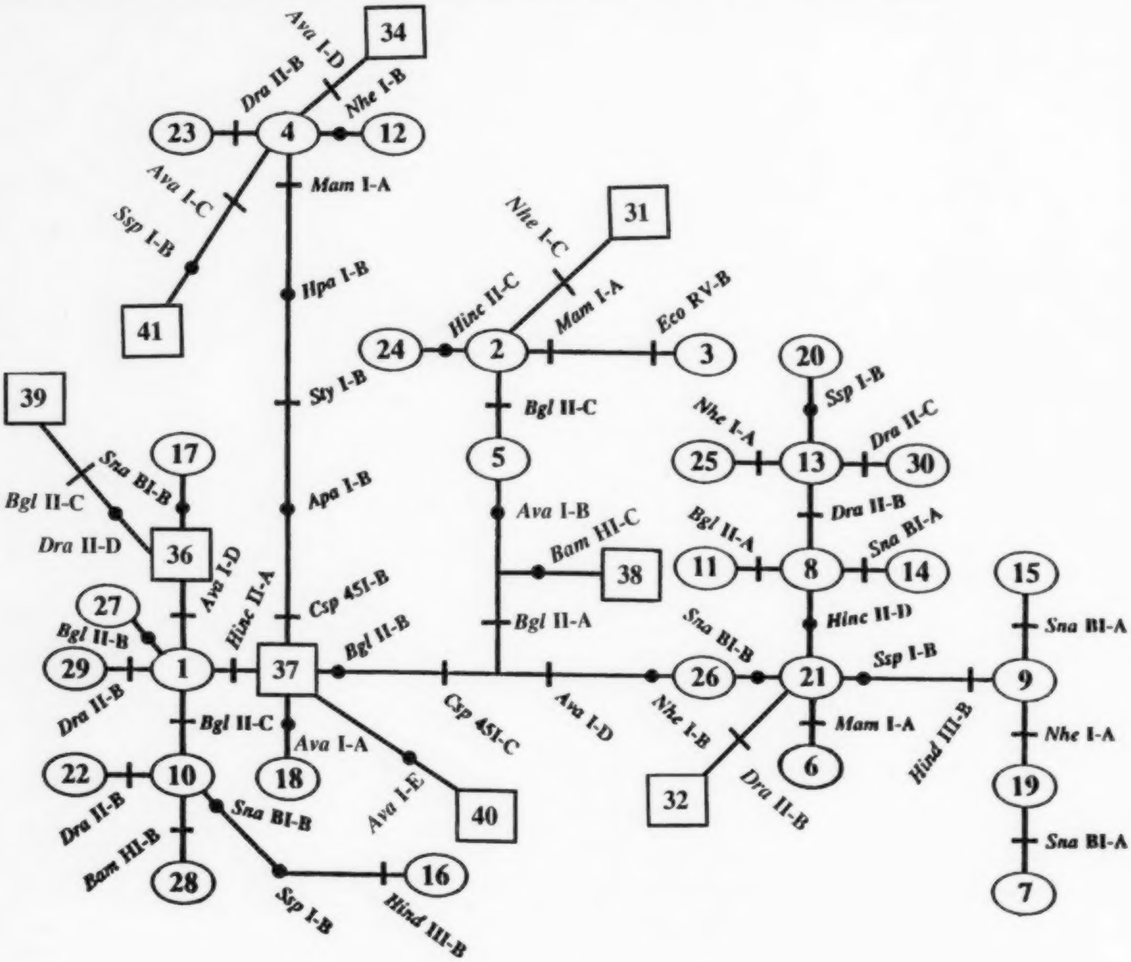
[2] A complete set of haplotype distribution data may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council of Canada, Ottawa, ON K1A 0S2, Canada.

Fig. 2. Wagner parsimony network of Danzmann et al. (1993) for rainbow trout haplotypes found in eastern North America, with the nine new haplotypes found in this study (squares) added. Site gains are indicated with a dot and losses with a bar, from clone 18.


34
Ava I-D
Dra II-B
Nhe I-B
23
4
12
Mam I-A
Ava I-C
Ssp I-B
41
31
Nhe I-C
Hpa I-B
Hinc II-C
Mam I-A
Eco RV-B
24
2
3
20
Sty I-B
Bgl II-C
Ssp I-B
39
5
Nhe I-A
Dra II-C
25
13
30
17
Sna BI-B
Bgl II-C
Dra II-D
Apa I-B
Ava I-B
Dra II-B
Bgl II-A
Sna BI-A
36
Bam HI-C
38
11
8
14
15
Ava I-D
Hinc II-A
Csp 451-B
Bgl II-A
Hinc II-D
27
Sna BI-A
Bgl II-B
Bgl II-B
Sna BI-B
Ssp I-B
29
1
37
26
21
9
Dra II-B
Bgl II-C
Csp 451-C
Ava I-D
Nhe I-B
Mam I-A
Hind III-B
Nhe I-A
Ava I-A
22
10
18
6
19
32
Dra II-B
Sna BI-B
Ava I-E
40
Dra II-B
Sna BI-A
Bam HI-B
28
16
7
Ssp I-B
Hind III-B

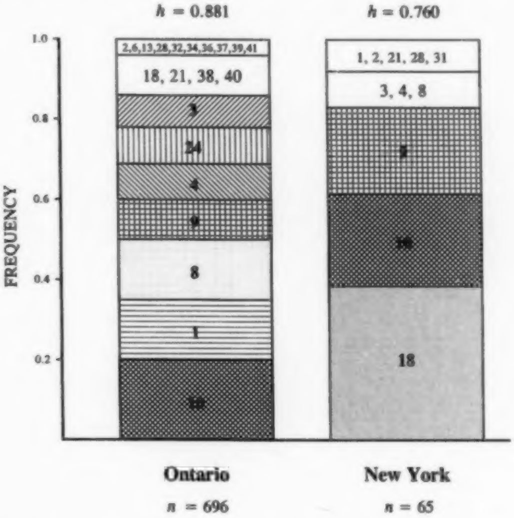
genome. The number of polymorphic sites ranged from 12 to 17, while the number of monomorphic sites totalled 123. Fifteen enzymes produced polymorphic patterns and 19 enzymes produced monomorphic patterns with 65 and 58 conserved sites, respectively (Danzmann et al. 1993; Ferguson et al. 1993). All enzymes had six-base recognition sequences, so 840 possible base pairs (bp) of approximately 16 780 bp were examined.

Inspection of overall haplotype distribution reveals a difference in genetic variation between fish from northern and eastern tributaries (Fig. 3). The 696 Ontario fish contained 21 haplotypes, with 7 haplotypes in frequencies higher than 5%, while the 65 New York fish included 12 haplotypes, with only 3 in higher frequency than 5%. This difference in haplotype distribution was statistically significant (χ^2, $P < 0.001 \pm 0.0$).

Nucleon diversity (h) was 0.885 for all fish pooled, 0.881 for Ontario fish, and 0.760 for New York fish. Among individual populations (Table 2), nucleon diversity was high and consistent (0.866–0.890) for Ontario samples. Similar levels of genetic variability were found in an initial 1989–1990 sampling of the Ganaraska population (Danzmann et al. 1993; Ferguson et al. 1993). However, nucleon diversity was lower and more variable (0.602–0.752) in New York samples. When population values were averaged, lakewide $h = 0.789$ ($n = 8$), Ontario $h = 0.867$ ($n = 5$), and New York $h = 0.658$ ($n = 3$). This difference in diversity between Ontario and New York population averages was statistically significant (t-test, $P < 0.01$, 6 df).

Partitioning of genetic variation, assessed with AMOVA for three types of population groupings, demonstrated definite subdivision at a lakewide level (Table 3). Populations were considered to be fish from each of the six drainages accessible to migration and the two stream sections isolated by barriers to gene flow. When each of the eight populations lakewide was considered a separate group, 3.7% of the total

Fig. 3. Distribution of haplotype frequencies in all Ontario (north shore) and New York (east shore) fish. Numbers in bars represent haplotype (clonal) designations. Unpatterned tops of bars show haplotypes that have frequencies <0.05. Nucleon diversity values (h) are give at top of bars.



variation was found among them (Table 3, part *a*). Partitioning was then compared for each of the two widely separated geographic areas (Table 3, part *b*). Only 0.5% of total genetic variation was found among the five Ontario populations, suggesting consideration of Ontario populations as a single region, since almost all variation resides within populations. In the three New York populations, however, a much larger proportion of the variation (19%) was found among populations, suggesting subdivision within this geographic area. A nested analysis on two hierarchical levels was then conducted for all eight populations (Table 3, part *c*), using the regional grouping that best explained subdivision at a lakewide level. This choice was based on maximal inter-region variance of potential groupings, suggested as a criterion by Excoffier et al. (1992) to define suprapopulation groups. When the populations were divided into all Ontario, New York migratory, and New York isolated regions, 13.9% of the total genetic variation was found among regions and 85.7% within populations, while only 0.4% can be attributed to among populations within regions. All probability values were significant.

The effectiveness of different samples sizes in adequately detecting diversity was calculated for Ontario and New York populations separately and compared with actual sampling results (Fig. 4). An "interim asymptote" in actual haplotype accumulation (Fig. 4, shaded bars) was reached by 20–25 fish, but a better representation of diversity was reached by 175–200 fish. Given the diversity observed, sample sizes of less than 20 were deemed inadequate for separate analyses and were therefore pooled. The equation for the fitted log curve of the actual data was $y = 3.49 \log_e(x) - 1.76$. Estimates of haplotype accumulation for sampling effort, plotted using equations for fitted log curves of Ontario and New York simulated data ($y = 3.42 \log_e(x) - 1.76$ and $2.74 \log_e(x) - 1.39$, respectively), revealed an inflection point in the Ontario curve (Fig. 4, solid line) between 50 and 75 fish, suggesting that future sampling should include at least 50 fish per population. This minimum sample size is consistent with recommendations for species with widely distributed common genotypes (reviewed by Carvalho and Hauser 1994).

Heterogeneity analysis

At the local hierarchical level, haplotype distribution was examined for spatial heterogeneity among sites within two Ontario streams: Ganaraska River and Shelter Valley Brook (see Fig. 1, inset). In the Ganaraska study, 12 haplotypes were found in 104 juveniles collected at four sites (Kendal, Osaca, Elizabethville, Canton) over 2 years. Since all annual sample sizes were <20 from each site, samples for both years were pooled by site to increase sample sizes for spatial heterogeneity testing among the sites. When all sites were tested together, the overall P value was 0.058, suggesting marginal heterogeneity within this drainage (Table 4). Further testing revealed one difference between pairwise comparisons (Elizabethville vs. Osaca, $P = 0.031$; table-wide α level for multiple tests = 0.0083).

In the local study for Shelter Valley Brook, 16 haplotypes were found among 197 juveniles collected at five sites (Isolated, West, East, Main, Lower) representing 2 years. Samples were first tested for temporal stability when both annual sample sizes were ≥ 20. No significant differences were found between years of the West and Main sites tested (Table 5A), so both years were pooled to increase sample sizes. Although heterogeneity was not found when all Shelter sites were tested together (overall $P = 0.440$), pairwise testing (Table 5B) revealed one difference (Main vs. Isolated, $P = 0.027$; table-wide α for multiple tests = 0.005).

At the regional level, haplotype distribution was examined among stream populations for Ontario and New York separately. In the Ontario study, 672 adult and juvenile fish sampled in 1992–1993 from migration-accessible sections of four streams (Wilmot, Ganaraska, Barnum, Shelter) were compared (Fig. 5). No significant spatial heterogeneity was detected when all populations were tested simultaneously (overall $P = 0.110$). When pairwise comparisons were made between streams (Table 6), the Ganaraska population was more different from its neighbours on the east (Barnum and Shelter, $P = 0.103$ and 0.111, respectively) and particularly the west (Wilmot, $P = 0.0098$, table-wide $\alpha = 0.008$), than the more widely separated populations (Wilmot and Shelter) were from each other ($P = 0.312$). The Wilmot–Ganaraska difference was due to juveniles ($P = 0.0004$, Wilmot $n = 68$, Ganaraska $n = 112$), and not adults ($P = 0.569$, Wilmot $n = 105$, Ganaraska $n = 89$).

Temporally, only one difference was found in haplotype distributions among collection periods and life-history types for Ontario migratory populations (Table 7). Shelter's 1992 and 1993 juveniles were different from each other ($P = 0.021$, table-wide $\alpha = 0.008$). Nucleon diversity also declined slightly but nonsignificantly during the study period in both Ganaraska (0.900–0.873) and Shelter (0.900–0.878) populations.

In the New York regional study, 65 juveniles sampled in 1993 from three streams (Little Sandy, Salmon, Prince) were

Table 2. Nucleon diversity values (*h*) for individual populations of rainbow trout from the Lake Ontario watershed.

Year	Stream	Sample type	*n*	*h*
Ontario				
1992–1993	Wilmot	Adults + juveniles	173	0.868
1992–1993	Ganaraska	Adults + juveniles	202	0.883
1993	Barnum	Adults	45	0.866
1992–1993	Shelter (migratory)	Adults + juveniles	252	0.890
1992	Shelter (isolated)	Juveniles	24	0.826
New York				
1993	Little Sandy	Juveniles	24	0.602
1993	Salmon River	Juveniles	21	0.752
1993	Prince (isolated)	Juveniles	20	0.621

Table 3. Analysis of molecular variance to partition genetic variation of Lake Ontario rainbow trout among and within populations and regions for all eight populations considered individually (*a*), for the two widely separated geographic areas (*b*), and at two hierarchical levels (population and region) in a nested analysis for three regions (*c*).

		Observed partition			
Grouping and variance components	df	Variance	% of total	*P*	Φ
(*a*) Individual populations					
Eight populations (five Ont, three NY)					
Among populations	7	0.016	3.7	<0.0002	0.037
Within populations	53	0.430	96.3	<0.0002	0.037
(*b*) Geographic areas					
Ontario (five populations: Wil, Gan, Bar, Shel (mig), Shel (isol))					
Among populations	4	0.002	0.5	<0.0002	0.005
Within populations	691	0.439	99.5	0.018	0.005
New York (three populations: Little Sandy, Salmon, Prince (isol))					
Among populations	2	0.077	19.0	<0.0002	0.190
Within populations	62	0.328	81.0	<0.0002	0.190
(*c*) Hierarchical levels					
Eight populations in three regions (all Ont, NY (mig), NY (isol))					
Among regions	2	0.070	13.9	<0.0002	0.139
Among populations or regions	5	0.002	0.4	0.033	0.004
Within populations	753	0.430	85.7	<0.0002	0.143

Note: Statistics were calculated using WINAMOVA 1.53 (Excoffier et al. 1992) and are based on frequencies of equidistant haplotypes (i.e., without inclusion of haplotype divergence data). *P*, probability of having a more extreme variance component and Φ-statistic than the observed values by chance alone, based on comparisons with 5000 permutations per run (Φ_{CT}, among regions; Φ_{SC}, among populations within regions; Φ_{ST}, within populations); mig, migratory; isol, isolated; Wil, Wilmot; Gan, Ganaraska; Bar, Barnum; Shel, Shelter; Ont, Ontario; NY, New York.

compared. The dominant presence of clone 18 (mean frequency 55%) is obvious in haplotype distributions for the two migratory populations (Fig. 6). The overall *P* value for the three populations combined was <0.001, indicating definite heterogeneity present (Table 8). Further testing revealed that the isolated Prince population was significantly different from the migratory Little Sandy ($P < 0.0001$, table-wide $\alpha = 0.017$) and Salmon ($P = 0.0002$, table-wide $\alpha = 0.025$) populations, although the latter two were not different from each other.

At the lakewide level, all migratory and isolated populations were tested for differences in haplotype distributions between north (Ontario) and east (New York) shores. The isolated Shelter population was included separately because of its segregated location, although it was not different than the migratory Shelter population ($P = 0.520$). The overall *P* value of <0.001 for the eight populations tested together demonstrated significant spatial heterogeneity. Furthermore, all pairwise comparisons between Ontario and New York populations were individually different (Table 9), and all remained significant when sequential Bonferroni corrections to α levels were applied.

Genetic similarity

A UPGMA phenogram based on haplotype frequencies of

Fig. 4. Haplotype (clonal) accumulation for sampling effort. Patterned bars represent actual accumulation for the entire study. Simulated accumulations (50 replications) are shown separately for Ontario (solid line) and New York fish (broken line). Predicted results from the log-curve equations are plotted by squares for Ontario and triangles for New York fish.

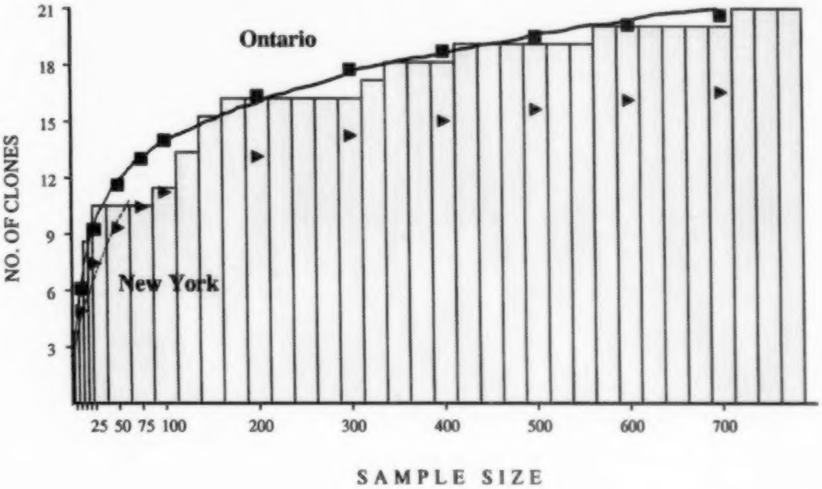


Table 4. Local level of hierarchical analysis for Ganaraska River rainbow trout, showing nucleon diversity values (*h*) and probability estimates (*P*) for pairwise comparisons of haplotype distributions between sites.

	Kendal	Osaca	Elizabethville	Canton
n	24	32	25	24
No. clones	7	9	8	10
h	0.848	0.871	0.833	0.895
Kendal	—	0.810±0.016	0.192±0.013	0.514±0.016
Osaca		—	0.031±0.002*	0.599±0.016
Elizabethville			—	0.491±0.016

Note: *P* values ± SEs are derived from Monte Carlo simulations. The overall *P* (four sites tested together) was 0.058.

*Statistically significant at $\alpha = 0.05$.

Lake Ontario rainbow trout indicated two clusters of naturalized populations (Fig. 7). A close relationship was found among all five 1992–1993 Ontario populations (especially Wilmot and Shelter migratory) and between the two New York migratory populations. However, the isolated New York population (Prince Brook) branched separately from the other two clusters.

Discussion

Although information on genetic population structure is now deemed crucial in resource management for many species in their native range (e.g., Pacific salmon on the American west coast), it has largely been ignored for introduced fishes. Our study is one of the few applications of mtDNA analysis to naturalized populations, documenting evidence for separate genetic structures developing among three regions along Lake Ontario's north and east shores. Previously, Chapman (1989) also demonstrated divergence with mtDNA among bluegill (*Lepomis macrochirus*) populations, but those populations were initiated from a single introduction and differentiation was likely governed by sexual asymmetry in life histories. In another mtDNA study on re-introduced lake trout (*Salvelinus namaycush*) strains, Grewe (1991) found differential reproductive success between two reef populations in Lake Ontario, which could eventually lead to geographic genetic structuring.

Study systems of introduced fish are also useful as contemporary models in ecological genetic research, since introductions and stocking are analogous to initial founder and dispersal events for colonizing species. Differential establishment and subsequent reproductive isolation in homing species such as salmonids could accelerate interpopulational divergence and local adaptation, resulting in multiple evolutionary trajectories. Monitoring the genetic dynamics of population structuring in naturalized species this way would

Table 5. Local level of hierarchical analysis for Shelter Valley Brook rainbow trout, showing nucleon diversity values (*h*) and probability estimates (*P*) for pairwise comparisons of haplotype distributions.

(A) Interannual variation within some sites.

	1992			1993			
	n	No. of clones	*h*	*n*	No. of clones	*h*	*P*
West	20	8	0.879	26	10	0.911	0.265±0.014
Main	32	11	0.879	27	9	0.837	0.250±0.014

(B) Spatial heterogeneity testing between sites.

	Isolated 1992	West 1992–1993	East 1992–1993	Main 1992–1993	Lower 1993
n	24	46	39	59	29
No. clones	8	12	12	13	12
h	0.826	0.898	0.900	0.861	0.919
Isolated	—	0.667±0.015	0.652±0.015	0.027±0.002*	0.153±0.011
West		—	0.579±0.016	0.291±0.014	0.573±0.016
East			—	0.649±0.015	0.664±0.015
Main				—	0.584±0.016

Note: *P* values ± SEs are derived from Monte Carlo simulations. The overall *P* value (five samples tested together) was 0.440.
*Statistically significant at $\alpha = 0.05$.

Fig. 5. Distribution of haplotype frequencies among Ontario migratory populations. Bar widths represent relative sample sizes for this figure. Numbers in bars are haplotype (clonal) designations. Unpatterned tops of bars group haplotypes that have frequencies of <0.05. Nucleon diversity values (*h*) are given at top of bars.

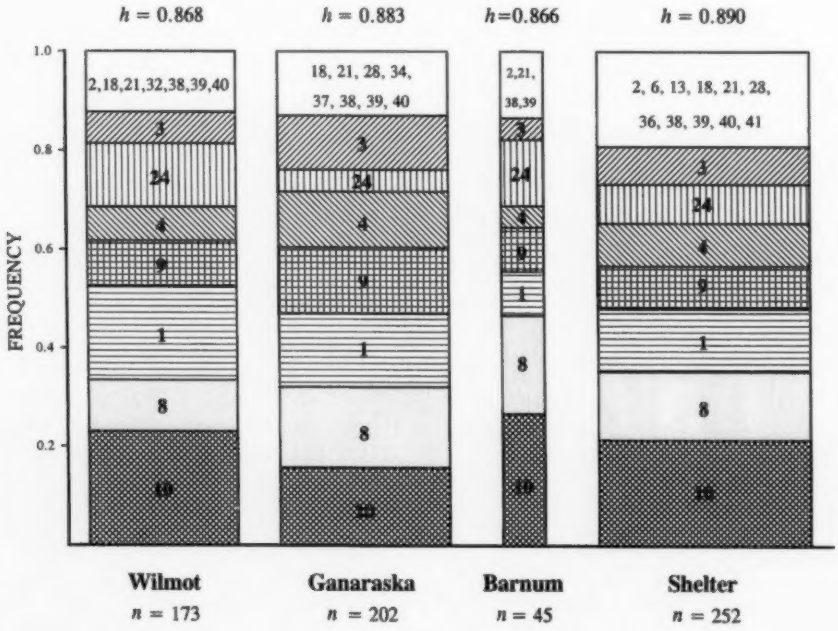


Table 6. Probability estimates (*P*) for pairwise comparisons testing spatial heterogeneity between Ontario populations of migratory rainbow trout.

	Wilmot	Ganaraska	Barnum	Shelter
n	173	202	45	252
No. clones	14	15	11	18
Wilmot	—	0.0098±0.0014*	0.265±0.014	0.312±0.015
Ganaraska		—	0.103±0.010	0.111±0.010
Barnum			—	0.615±0.015

Note: All samples were from 1992–1993 except Barnum (1993 only). *P* values ± SEs are derived from Monte Carlo simulations. The overall *P* value (four samples taken together) was 0.110.

*Statistically significant at $\alpha = 0.05$.

Table 7. Nucleon diversity values (*h*) and probability estimates (*P*) for pairwise comparisons testing temporal heterogeity between collection groups of Ontario migratory populations.

	Spring 1992 adults	Fall 1992 adults	Spring 1993 adults	1992 juveniles	1993 juveniles
Wilmot					
n	66	23	16	68	na[a]
h	0.872	0.870	0.849	0.871	na
Spring 1992 adults	—	0.544±0.016	nd[b]	0.275±0.014	na
Fall 1992 adults		—	nd	0.801±0.013	na
Ganaraska					
n	50	28	12	58	54
h	0.900	0.868	0.894	0.875	0.873
Spring 1992 adults	—	0.478±0.016	nd	0.374±0.015	0.441±0.016
Fall 1992 adults		—	nd	0.535±0.016	0.125±0.011
1992 juveniles				—	0.412±0.016
Shelter					
n	42	7	30	69	104
h	0.900	0.904	0.904	0.893	0.878
Spring 1992 adults	—	nd	0.687±0.015	0.413±0.016	0.581±0.016
Spring 1993 adults			—	0.653±0.015	0.104±0.010
1992 juveniles				—	0.021±0.002*
All					
n	158 (W–G–S)	58 (W–G–S)	103 (W–G–B–S)	195 (W–G–S)	158 (G–S)
h	0.884	0.865	0.885	0.886	0.876
Spring 1992 adults	—	0.353±0.015	0.735±0.014	0.824±0.012	0.107±0.010
Fall 1992 adults		—	0.289±0.014	0.232±0.013	0.149±0.011
Spring 1993 adults			—	0.893±0.010	0.196±0.013
1992 juveniles				—	0.070±0.008

Note: *P* values ± SEs are derived from Monte Carlo simulations. The overall *P* values were 0.729±0.014 for Wilmot Creek (three samples), 0.257±0.014 for Ganaraska River (four samples), 0.139±0.011 for Shelter Valley Brook, and 0.422±0.016 for all samples (five samples). W, Wilmot; G, Ganaraska; B, Barnum; S, Shelter.

[a] na, not available (no sample taken).

[b] nd, not done because of low *n*.

*Statistically significant at $\alpha = 0.05$.

Fig. 6. Distribution of haplotype frequencies among New York populations. Bar widths represent relative sample sizes for this figure. Numbers in bars designate haplotypes (clones). Unpatterned tops of bars group haplotypes that have frequencies of <0.05 Nucleon diversity values (*h*) are given at top of bars.

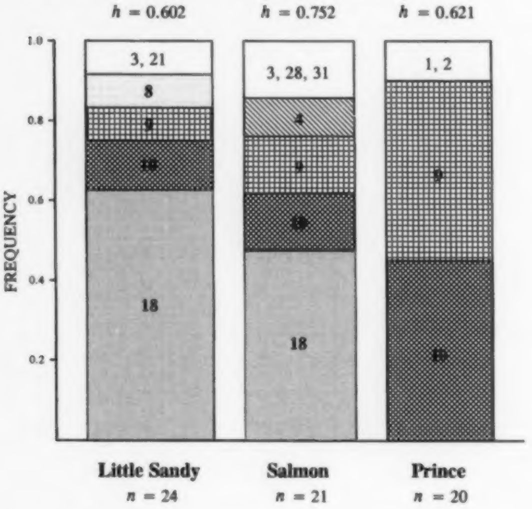


Table 8. Probability estimates (*P*) for pairwise comparisons testing spatial heterogeneity between New York populations of rainbow trout.

	Little Sandy	Salmon	Prince
n	24	21	20
No. clones	6	7	4
Little Sandy	—	0.457±0.016	0±0
Salmon		—	0.0002±0.0002*

Note: *P* values ± SEs are derived from Monte Carlo simulations. Little Sandy and Salmon populations are accessible to lakeward migration, but the Prince population is isolated. The overall *P* value (three samples tested together) was 0.

*Statistically significant at α = 0.05.

facilitate examination of the interplay between ecological and evolutionary processes (Ford 1975; Carvalho 1993; Real 1994).

Differences in the genetic character of naturalized populations from Lake Ontario have obviously developed during the last century from an admixture of multiple introduction sources. Present genetic distributions are, therefore, some function of several factors: strains, numbers, and locations stocked; imprinting success for homing fidelity; founding opportunities; survival; reproductive success; number of generations elapsed; effective population size; gene flow; and drift. Although the mitochondrial genome has long been considered selectivity neutral (Moritz et al. 1987), recent work on *Drosophila* indicates that evolution in both the mitochondrial and nuclear genomes is governed by many of the same selective forces (Ballard and Kreitman 1994). Recent studies on domestic rainbow trout, in fact, have demonstrated correspondence between specific haplotypes and spawning time (Ferguson et al. 1993; Danzmann et al. 1994), so selection could potentially be another influencing process.

In addition to a major lakewide division of migratory populations, the New York fish were further substructured into two groups, isolated and migratory, which differed independently from all Ontario populations. The distinguishing features of the two New York groups were even fewer haplotypes in the isolated population and predominance of clone 18 in migratory populations as a nearly unique stock-specific marker. Limited gene flow, harsher ecological conditions, and smaller effective population size could explain lower diversity in the isolated New York population. Migratory populations in New York, however, have been exposed to much more stocking recently than in Ontario and are largely composed of one haplotype, clone 18. This haplotype also occurs at very high frequency in the main stocking strain, Chambers Creek, currently used by New York (Danzmann et al. 1993; Dueck 1994). If the preponderance of naturalized clone 18 fish can truly be attributed to recent massive stocking, there are two major implications. First, our results indicate successful secondary colonization and strong female homing fidelity by this strain in eastern New York streams. Second, consequently this management strategy has likely altered the trajectory of genetic population structuring for rainbow trout in that region by swamping primary colonizers with a single haplotype, thus homogenizing previously established genetic architecture.

Two reasons (length of isolation time and potential stocking differences) may account for the genetic differentiation between isolated and migratory populations found in New York but not in Ontario. New York's Prince Brook, separated from the lower Salmon River by a natural 120 foot (1 foot = 0.3048 m) waterfall and two dams, has been stocked with non-anadromous forms since the late 1800s (L. Wedge, NYDEC, personal communication). Ontario's upper west Shelter, isolated by a dam in the 1920s prior to any reports of rainbow trout in the area, has only been stocked during the last 20 years with spawning migratory fish from below the dam (D. Bell, OMNR, personal communication).

Explanations for the subtle differences among migratory populations from Ontario streams are less straightforward, since most genetic variation resides within populations. For example, the same seven high-frequency haplotypes are shared by all four groups although proportions differ among them, while private haplotypes account for only 0–2.2% of the variation in any one drainage. Under these circumstances, our results would have less credence if sample sizes were smaller because of the likelihood of sampling error. However, analysis of sampling effectiveness for this situation and large samples sizes of 173–252 in major drainages provide more confidence in our findings.

Regional patterning among Ontario migratory populations does not show the geographic–genetic relationship predicted from a stepping-stone model of population structure expected when founding opportunities are comparable (May and Krueger 1990). Instead, the rather disjunct nature of Ganaraska haplotype distribution is most evident when compared with the Wilmot population. As the Ganaraska population also differed from its other neighbours, Barnum and Shelter, more than they differed from Wilmot, it appears that

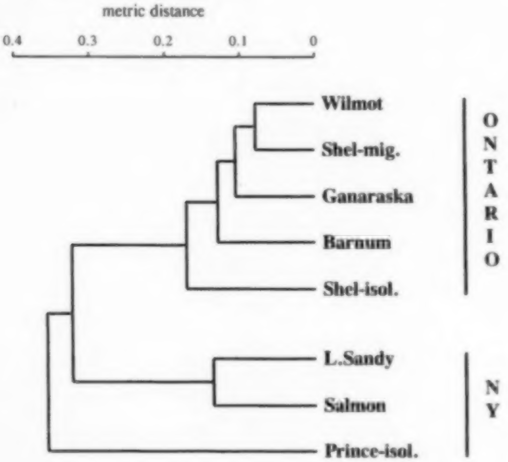
Table 9. Probability estimates (*P*) for pairwise comparisons of haplotype distributions testing spatial heterogeneity between rainbow trout populations from north and east shores of Lake Ontario.

	New York		
	Little Sandy	Salmon	Prince
n	24	21	20
No. clones	6	7	4
Ontario			
Wilmot (*n* = 173, 14 clones)	0±0*	0±0*	0.008±0.003*
Ganaraska (*n* = 202, 15 clones)	0±0*	0±0*	0.004±0.002*
Barnum (*n* = 45, 11 clones)	0±0*	0±0*	0.008±0.003*
Shelter, migratory (*n* = 252, 18 clones)	0±0*	0±0*	0.029±0.005*
Shelter, isolated (*n* = 24, 8 clones)	0±0*	0±0*	0±0*

Note: *P* values ± SEs are derived from Monte Carlo simulations. The overall *P* value (eight samples tested together) was 0.

*Statistically significant at $\alpha = 0.05$.

Fig. 7. UPGMA phenogram of relationships among populations of Lake Ontario rainbow trout, based on Manhattan metric distances between populations from haplotype frequency differences (distance shown is 0.5 D_{M}).



the Ganaraska (middle) population is anomalous. However, such as difference might be expected considering that upstream spawning habitat has only been available within the last 20 years, provided by fishway construction circumventing a lower Ganaraska dam. By contrast, fish in neighbouring drainages have had unlimited upstream access since rainbow trout were first seen in the area 45 years ago. As a younger population, Ganaraska's genetic profile could still be stabilizing since the difference with Wilmot was only significant in juveniles, although no temporal change between age groups in either population was detected. It is also probable that the Ganaraska population developed in part because of straying from neighbouring streams just after lamprey control and stocking were initiated (reviewed by Dueck 1994). Therefore, the consequent population expansion may have magnified chance differences in haplotype frequencies of new colonizers. In spite of adequate establishment time and homing capacity, differentiation among Ontario populations may have been limited by the counteracting effect of gene flow (resulting from demographic instability following secondary range expansion and increased survival) on stochastic lineage extinction from selection and drift (Avise et al. 1984; Slatkin 1987). This may also account for the higher diversity levels detected among the Ontario populations.

Population structuring was minimal on a local level in the two Ontario streams surveyed where one difference between sites was found within each population. Given the high level of diversity in Ontario fish and the small sample sizes, it is quite possible that these differences could be due to sampling error. However, it is also possible that differentiation could be occurring among small branches because of limited access over a short time. For example, although small sample size precluded consideration in our results, the 2 years of Shelter East data differed significantly in haplotype distribution ($P = 0.001$) and in nucleon diversity (0.919 vs. 0.818), probably because a new beaver dam blocked access for parents of the second year's samples (personal observation). This is precisely the sort of ecological event that could induce stochastic lineage extinction in a small branch and thus development of local structuring.

Mitochondrial diversity of naturalized rainbow trout in the Lake Ontario watershed is noticeably higher (lakewide $h = 0.885$) than in most natural populations of freshwater fish or in hatchery populations that have been comparably studied. For example, overall *h*s of 0.766, 0.653, and 0.550 were found in lake trout (*Salvelinus namaycush*) by Grewe and Hebert (1988), lake whitefish (*Coregonus clupeaformis*) by Bernatchez and Dodson (1991), and walleye (*Stizostedion vitreum*) by Billington and Hebert (1988), respectively. In one of the first RFLP studies on native rainbow trout and steelhead, Wilson et al. (1985) detected 8 haplotypes among 38 samples in 8 populations (*h* was undeterminable from data presented), lower than that predicted in our study. The variability found among Lake Ontario rainbow trout contrasts with that of domestic strains maintained in Ontario aquaculture situations where *h* averaged 0.423 (Ferguson et al. 1993).

Both native populations and hatchery strains are often characterized by a few major haplotypes (Bernatchez and Dodson 1991; Billington and Hebert 1988; Ferguson et al. 1993). Native populations may also be dominated by unique haplotypes in different regions and usually also contain several rare types (Billington and Hebert 1991). Therefore, it might be expected. that, when rainbow trout from multiple sources were introduced into Lake Ontario, a higher genetic diversity would result than occurs in nature.

The high nucleon diversity found for Lake Ontario rainbow trout is attributable not only to many haplotypes but also to relatively equitable distribution among them, especially in fish from Ontario's north-shore streams. This diversity pattern is actually more like that of marine species with good dispersal (reviewed by Grewe et al. 1994) than of freshwater species with barriers to gene flow or even established anadromous populations semi-isolated by homing (Gyllensten 1985; Ward et al. 1994). High, even diversity probably originated from the "layering" of introductions and establishment of strains from several different sources throughout the history of Lake Ontario stocking. Avise et al. (1984) showed that stochastic lineage extinction was dramatically slowed in expanding populations. This might explain why high diversity has been maintained in Ontario populations, which were provided expansion opportunities by lamprey control and the Ganaraska fishway, despite discontinuation of stocking in the area 20 years ago.

Since genetic differentiation was evident at the lakewide level and between New York migratory and isolated populations, adaptations of naturalized breeding populations to local conditions have likely developed over the last century. The potential existence of localized adaptations has important implications for management strategies and would suggest that these reproductively distinct units be managed separately. As segregation among north-shore Ontario populations could not be disproved, provincial management plans should factor in migratory and reproductive behaviour in addition to genetic data (May and Krueger 1990). The existence of genetically distinct, self-sustaining naturalized populations suggests that continued stocking, particularly with monotypic strains, should be abandoned in favour of other strategies such as water quality improvement and steam habitat restoration that would enhance local reproductive success. More stringent harvest control might also be considered if Lake Ontario rainbow trout are in post-irruptive decline, a pattern suggested by the Riney–Caughley population dynamics model for introduced species (Hartman 1994).

Our conclusions regarding genetic population structure are based solely on evidence from the mitochondrial genome and have inherent limitations. The nonrecombinant, maternal transmission of mtDNA makes it equivalent to a single locus with multiple alleles (Slatkin and Maddison 1989; Avise 1994). Population structure may be more accurately characterized by examination of multiple loci, preferably with complementary modes of transmission (Ferguson 1990), particularly since mtDNA can only indicate female-moderated gene flow and population structure. Although no gender bias is known for homing in *Oncorhynchus* species (T.P. Quinn, School of Fisheries, University of Washington, Seattle, Wash., personal communication), male-biased straying has been suggested in other fish species (Billington and Hebert 1988). If gender biases in philopatry or straying do occur, estimates of genetic structures based on mtDNA data may not be fully representative (Bermingham 1990). Further analyses utilizing nuclear markers could complement our results by providing more loci for enhanced resolution of interpopulation divergence and enable detailed examination of gender symmetry in gene flow.

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The dynamics of Baltic fish stocks on the basis of a multispecies stock–production model

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Abstract: A multispecies stock–production model was developed and used to estimate the dynamics of Baltic cod (*Gadus morhua callarias*), herring (*Clupea harengus*), and sprat (*Sprattus sprattus*) stocks from 1982 to 1992. Inputs to the model were estimates of the fishing effort, recruitment indices, mean weight of fish in the stock, von Bertalanffy's growth parameters, and residual natural mortality. The model's parameters that were not known in advance (e.g., food preference parameters, catchabilities, calibration coefficient for recruitment indices) were estimated by minimizing the deviations of observed catches and food composition of cod from the values arrived at from the model. The standard errors of the fitted parameters of the model were evaluated using a bootstrap procedure. Sensitivity analyses showed that growth parameters had the largest influence on the model outcome. The model produced estimates of the population dynamics of Baltic fish stocks consistent with the estimates from age-structured models. In the period analyzed, the biomass of cod decreased by about 80%, the biomass of herring was stable, and sprat biomass fluctuated, increasing finally to a record high level. The estimated decrease of cod biomass is reflected in a decreasing predation mortality of herring and sprat.

Résumé : Nous avons élaboré un modèle multispécifique de la production des stocks et utilisé ce modèle pour évaluer la dynamique des stocks de morue (*Gadus morhua callarias*), de hareng (*Clupea harengus*) et de sprat (*Sprattus sprattus*) de la mer Baltique de 1982 à 1992. Le modèle comportait les intrants suivants : estimations de l'effort de pêche; indices de recrutement; poids moyen des poissons de chaque stock; paramètres de croissance de von Bertalanffy; et mortalité naturelle résiduelle. Nous avons estimé les paramètres du modèle qui n'étaient pas connus à l'avance (paramètres liés aux préférences alimentaires, potentiels de capture, coefficient de calibration des indices de recrutement, etc.) en réduisant au minimum les écarts entre valeurs observées et valeurs prédites par le modèle, en ce qui concerne les prises de morue et la composition du régime alimentaire de cette espèce. Nous avons utilisé une méthode de type bootstrap pour évaluer les erreurs types caractérisant les paramètres rajustés du modèle. Des analyses de sensibilité ont montré que ce sont les paramètres de croissance qui influent le plus sur les résultats obtenus au moyen du modèle. En ce qui concerne la dynamique des populations de poisson de la Baltique, le modèle a permis des estimations compatibles avec celles provenant de modèles axés sur l'âge. Au cours de la période d'analyse, la biomasse de la morue a diminué d'environ 80%, celle du hareng est demeurée stable, tandis que celle du sprat a fluctué puis finalement atteint un niveau record. La diminution observée dans le cas de la morue s'est traduite, chez le hareng et le sprat, par une baisse de la mortalité imputable à la prédation.
[Traduit par la Rédaction]

Introduction

In the past two decades, there has been a rapid development of age-structured multispecies models. This occurred because intensively developing fisheries brought about overfishing of many exploited fish stocks, which in turn may have affected interspecific interactions. It thus became advisable to take into account the impact of predator stocks on prey. Many authors have contributed to the development of the age-structured multispecies models, with the most comprehensive being that of Andersen and Ursin (1977), which is a complex model of the ecosystem. However, the most popular and best tested model is the multispecies virtual population analysis (MSVPA) (Helgason and Gislason 1979; Pope 1979; Sparre 1980).

On the other hand, less progress has been made in the development of multispecies production models. An example of such a model is the multispecies model of Schaefer (Larkin 1966; Pope 1976; Sullivan 1991). Production models, although theoretically less realistic than the age-structured models, are valuable because they do not require as much data as the age-structured models. Biomass-based production models use catch statistics (catches, fishing effort), and there is no need to determine the age structure of a population. Hence, these models are a useful assessment tool in cases when it is difficult to determine the age of fish. Horbowy (1992*b*) developed a multispecies stock–production model based on the age structured model of Andersen and Ursin (1977). The model, in which predation mortality is dependent on the biomass of predators, food supply, food requirements of predators, and suitability index of prey to predator, is a multispecies extension of the single species model presented by Horbowy (1992*a*). In the model, the biomass of prey is

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influenced by the predator stock, but the prey stock does not influence the dynamics of the predator stock as it does in the Schaefer multispecies model. The advantage of this approach is that some of the model parameters, having specific biological meaning, can be estimated outside the model.

The model has been tested in two ways. First (Horbowy 1992*b*), a system of three populations, one of which was a predator and two of which were prey, was generated using the Andersen and Ursin (1977) equations and assumed parameter values. Next, the parameters of such a system were estimated using the developed multispecies stock–production model. The model reflected the generated biomass with a mean error of about 10%. The second test of the model (Horbowy 1992*b*) was its application to estimate the dynamics of Baltic cod (*Gadus morhua callarias*), herring (*Clupea harengus*), and sprat (*Sprattus sprattus*) in the Baltic Sea for 1977–1990. However, the results depended on parameter estimates obtained by virtual population analysis (VPA), namely, fishing mortality and recruitment figures that were treated as the indices of the fishing effort and recruitment to the populations, respectively. In this way, one of the most difficult points in fitting the model was avoided and the question of the model's performance with respect to that of age-structured models has not been answered.

The goal of this paper is to use the model to estimate the dynamics of Baltic cod, herring, and sprat based on survey and fishing effort data to make its results independent of VPA results. In addition, data on cod stomach contents (Sparholt 1993) are used when fitting the model and the bootstrap method (Efron 1982) is applied to derive standard errors of the parameter values estimated within the model.

Methods

1. The multispecies production model

The basic equations of the age-structured multispecies model of Andersen and Ursin (1977) were a starting point in the derivation of the multispecies stock–production model. Let us assume that recruitment to a population is discrete, and let's develop the formula for the rate of change of population biomass in between recruitment events. The biomass of age group a of population s in time t may be presented as

$$B_{as}(t) = N_{as}(t)w_{as}(t)$$

where N is abundance and w is weight. Hence

$$\frac{\mathrm{d}B_{as}}{\mathrm{d}t} = w_{as}\frac{\mathrm{d}N_{as}}{\mathrm{d}t} + N_{as}\frac{\mathrm{d}w_{as}}{\mathrm{d}t}$$

Substituting $\mathrm{d}N/\mathrm{d}t$ and $\mathrm{d}w/\mathrm{d}t$ in the above formula with the corresponding formulae from the Andersen and Ursin model (1977), performing certain algebraic transformations, and summing $\mathrm{d}B_{as}/\mathrm{d}t$ over age (see Appendix), we obtain the formula:

$$(1)\qquad \frac{\mathrm{d}B_s}{\mathrm{d}t} = \left(\upsilon_s h_s \bar{w}_s^{-1/3} - q_s E_s - M1_s - k_s - \sum_{r=1}^{n} h_r \bar{w}_r^{-1/3} \frac{G_r^s B_r}{\sum_{i=1}^{n} G_r^i B_i + \mathrm{OT}} \right) B_s$$

where υ, h, and k are parameters of the von Bertalanffy's growth equation generalized by Andersen and Ursin (1977), where υ is the fraction of eaten food assimilated for growth; E is fishing effort; q is catchability coefficient; $M1$ is coefficient of natural mortality caused by reasons other than predation; $\bar{w}$ is mean weight of fish in the population; G_r^s is suitability of prey s to predator r; OT is other food; s and r are populations; and n is the number of populations.

Assuming the term in parentheses is constant or has a small variability in the time interval $(t, t + \mathrm{d}t)$, eq. 1 has the following approximate solution:

$$(2)\qquad B_s(t + \mathrm{d}t) = B_s(t)\exp(a_s(t)\,\mathrm{d}t)$$

where

$$a_s(t) = \upsilon_s h_s \bar{w}_s^{-1/3} - q_s E_s - M1_s - k_s - \sum_{r=1}^{n} h_r \bar{w}_r^{-1/3} \frac{G_r^s B_r}{\sum_{i=1}^{n} G_r^i B_i + \mathrm{OT}}$$

If recruitment takes place at time $t+\mathrm{d}t$, eq. 2 will assume the following form:

$$(3)\qquad B_s(t + \mathrm{d}t) = B_s(t)\exp[a_s(t)\,\mathrm{d}t] + R_s$$

where R is the biomass of the year-class recruited to the population. A similar approach in which the biomass of a population also changes continuously between discrete recruitment events was presented by Deriso (1980) for a single species model.

2. Input data to the model

The Baltic Sea has a relatively simple system of trophic levels, which facilitates the modelling of the multispecies interactions. The main predator is cod, which feeds mainly on herring, sprat, and invertebrates. Cod also eat gobies, sandeels, and rocklings, but these constitute only a small percentage of the cod diet (Zalachowski 1985; Uzars 1989). Salmonid fishes also eat herring and sprat, but their biomass is small compared with cod biomass and they may be omitted from the model. Similarly, birds and marine mammals have very limited influence on the predation mortality of herring and sprat. Thus, in the model we will estimate interactions among man (catches), cod, herring, sprat, and a component called "other food." The fishery operates on cod, herring, and sprat while cod prey on herring, sprat, and other food. The other food is composed of invertebrates and fish of minor importance. The model is restricted to mature fish, i.e., cod no younger than 3 years and herring and sprat no younger than 2 years.

Two stocks of the Baltic cod can be distinguished: the stock inhabiting waters east of Bornholm and the stock inhabiting waters west of Bornholm. We will apply the model to the larger eastern Baltic cod stock and herring and sprat stocks living east of Bornholm (International Council for the Exploration of the Sea (ICES) Subdivisions 25–32).

The time period for which we estimate the species' in-

Table 1. Values of input parameters to the multispecies production model.

Parameter	Cod	Herring	Sprat
H^a	0.968	2.84	5.13
SE (H)	0.097	0.26	0.47
k^a	0.46	0.57	2.00
SE (k)	0.05	0.067	0.20
$M1^b$	0.2	0.2	0.2
$w0^c$	437	26.5	10.0

[a] H and k, von Bertalanffy growth equation parameters ($H = vh$).

[b] $M1$, coefficient of residual natural mortality.

[c] $w0$, mean weight (g) of fish recruited to the population.

teractions is highly dependent upon the availability of data. For Baltic stocks of herring and sprat, the determination of fishing effort and recruitment indices involves some difficulty. For cod, standardized fishing effort data by country extend back to 1982. Recruitment indices for cod from international surveys of young fish are also available for this period. For herring and sprat, neither standardized fishing effort nor international young fish survey data are available. However, international acoustic surveys on herring and sprat provide some data on stock biomass and stock numbers at age beginning in 1982 (Anonymous 1991, 1992*a*, 1993). Accordingly, we will apply our model to 1982–1992.

The values of some parameters assumed to be known in the model are presented in Table 1.

The parameters of the von Bertalanffy equation for cod were taken from Horbowy (1992*a*), and the parameters for herring and sprat were taken from Horbowy (1992*b*). The v value of 0.2 was adopted for cod, which is the average v at age calculated in Anonymous (1992*b*). The level of the coefficient of residual natural mortality was assumed as in Anonymous (1992*b*).

Fishing effort for cod was estimated on the basis of standardized fishing effort by country (Anonymous 1994*a*), presented in a relative way (fishing effort in 1982 equals 1 for each country). An overall index of fishing effort was arrived at as the sum of products of national indices and national catches in weight. To estimate the indices of E exerted on herring and sprat, the formula

$$E = \frac{\text{Yield}}{\text{Acoustic biomass}}$$

was used. Assuming that acoustic estimates of herring and sprat biomass (for October) are not very different from mean yearly values, the above formula should provide an approximate index of fishing effort.

The mean weights, $\bar{w}$, for sprat and herring were calculated by dividing the stock biomass by its abundance in numbers, both estimated during the acoustic survey (Anonymous 1991, 1992*a*, 1993). For cod, mean weights were obtained as the averages of weight at age weighted by catch in numbers at age.

Recruitment in the model

The biomass of recruitment is determined in the model according to formula:

$$R = bIw0$$

where b is an unknown parameter, I is the index of year-class strength, and $w0$ is the weight of recruits taken from Anonymous (1994*a*, 1994*b*).

The index of cod year-class strength was taken from Anonymous (1994*a*). It is based on data from the international survey on young fish, smoothed by the generalized linear model. For herring, the Polish young fish survey index of recruitment was applied (Anonymous 1994*b*). This index refers to coastal spring spawning herring in the Gulf of Gdansk. The index of year-class strength of sprat was taken from the Latvian acoustic estimates of young sprat in Subdivisions 26 and 28, as this survey covered shallow waters with concentrations of young sprat (Anonymous 1994*b*).

3. Parameters estimated within the model

The initial biomass values ($B0$), suitabilities (G), catchability coefficients (q), coefficients (b) connected with population recruitment, and biomass of other food (OT) were estimated within the model. The usual way of doing this is to choose parameter values that minimize the squared deviations of the modelled catches from the observed catches.

As there are data on food composition in Baltic cod, one may include an additional term in the objective function to be minimized. We may determine in the model the ratio of herring biomass to the sprat biomass consumed by cod, $\widehat{\mathrm{BC}}_{\mathrm{hr}}/\widehat{\mathrm{BC}}_{\mathrm{sp}}$, and then include the deviations of this ratio from the observed ratio, $\mathrm{BC}_{\mathrm{hr}}/\mathrm{BC}_{\mathrm{sp}}$, in the minimized function of deviations of the model magnitudes from the observed ones. The observed mean ratio of the consumed herring biomass and sprat biomass was calculated on the basis of data from Sparholt (1993). These data present detailed quarterly-based food composition of cod, stratified by age groups of cod and its prey. First, to determine annual consumption of herring and sprat by adult cod, consumption by quarters was added in cod age groups. Then the total consumption of herring and sprat was calculated as the sum of consumption by age multiplied by the index of cod abundance at age and cod ration at age (the ration was assumed to be proportional to weight$^{2/3}$ (Andersen and Ursin 1977)). For the index of cod abundance, the catch at age in numbers was taken (Anonymous 1994*a*). This procedure was adopted for data from 1982 and 1987, as these years represented the highest number of analyzed stomachs collected in the first and second halves of the time period under consideration. In addition, the estimated ratio of herring and sprat consumed to other food consumed by cod, $(\widehat{\mathrm{BC}}_{\mathrm{hr}} + \widehat{\mathrm{BC}}_{\mathrm{sp}})/\widehat{\mathrm{OT}}$ was calculated for 1982 and incorporated into the minimization. Initial biomass, $\widehat{B0}$, was also included in the objective function. The observed $B0$ of herring and sprat in 1982 were taken from the acoustic estimates (Anonymous 1991), and the $B0$ value for cod was adopted from Horbowy (1992*a*).

In summary, the parameters $\hat{G}$, $\hat{q}$, $\hat{b}$, $\widehat{B0}$, and $\widehat{\mathrm{OT}}$ were found by minimizing the objective function

$$\begin{aligned}
&\mathrm{SS}(G_{\mathrm{c}}^{\mathrm{hr}}, G_{\mathrm{c}}^{\mathrm{sp}}, q_{\mathrm{c}}, q_{\mathrm{hr}}, q_{\mathrm{sp}}, b_{\mathrm{c}}, b_{\mathrm{hr}}, b_{\mathrm{sp}}, B0_{\mathrm{c}}, B0_{\mathrm{hr}}, B0_{\mathrm{sp}}, \mathrm{OT}) \\
&= \sum_t (\lambda_1(\ln\ Y_{\mathrm{c},t} - \ln\ \hat{Y}_{\mathrm{c},t})^2 + \lambda_2(\ln\ Y_{\mathrm{hr},t} - \ln\ \hat{Y}_{\mathrm{hr},t})^2 \\
&+ \lambda_3(\ln\ Y_{\mathrm{sp},t} - \ln\ \hat{Y}_{sp,t})^2) \\
&+ \lambda_4((\ln(\mathrm{BC}_{\mathrm{hr}}/\mathrm{BC}_{\mathrm{sp}})_{1982} - \ln(\widehat{\mathrm{BC}}_{\mathrm{hr}}/\widehat{\mathrm{BC}}_{\mathrm{sp}})_{1982})^2 \\
(4)\qquad &+ (\ln(\mathrm{BC}_{\mathrm{hr}}/\mathrm{BC}_{\mathrm{sp}})_{1987} - \ln(\widehat{\mathrm{BC}}_{\mathrm{hr}}/\widehat{\mathrm{BC}}_{\mathrm{sp}})_{1987})^2) \\
&+ \lambda_5(\ln((\mathrm{BC}_{\mathrm{hr}} + \mathrm{BC}_{\mathrm{sp}})/\mathrm{OT})_{1982} \\
&- \ln((\widehat{\mathrm{BC}}_{\mathrm{hr}} + \widehat{\mathrm{BC}}_{\mathrm{sp}})/\widehat{\mathrm{OT}})_{1982})^2 \\
&+ \lambda_6(\ln B0_{\mathrm{c}} - \ln\ \widehat{B0}_{\mathrm{c}})^2 + \lambda_7(\ln\ B0_{\mathrm{hr}} - \ln\ \widehat{B0}_{\mathrm{hr}})^2 \\
&+ \lambda_8(\ln\ B0_{\mathrm{sp}} - \ln\ \widehat{B0}_{\mathrm{sp}})^2
\end{aligned}$$

where $\hat{Y}$ is model catch, Y is observed catches, and subscripts c, hr, and sp refer to cod, herring, and sprat, respectively. The parameter λ represent statistical weights, being the inverse of variance associated with successive terms. However, these variances are not known a priori. We know only that the acoustic estimates have relatively large variance for the Baltic fish stocks. Therefore, for these analyses, it is assumed that all variances are equal to 1 except for the variances of the initial biomass, which are arbitrarily assumed to be two times higher. Parameter G is determined relative to a constant multiplier, so assuming, e.g., $G_{\mathrm{c}}^{\mathrm{sp}} = 1$, we have only to determine $G_{\mathrm{c}}^{\mathrm{hr}}$.

The standard errors of the $\hat{G}$, $\hat{q}$, $\hat{b}$, $\widehat{B0}$, and $\widehat{\mathrm{OT}}$ were determined using a bootstrap procedure (Efron 1982) based on 200 bootstrap replicates of resampled residuals.

4. Sensitivity analysis

An ordinary sensitivity analysis and an extended stochastic sensitivity analysis (Majkowski et al. 1981) were conducted. In the ordinary sensitivity analysis, the reaction of the estimated biomass to changes in the values of the input parameters ($v, h, k, M1, q, b, B0, G$, and OT) of the model by 5, 10, 20, and 50% were studied. In the extended stochastic analysis, it was assumed that these input parameters have approximately normal distributions. Standard errors for v and $M1$ were not known; so it was arbitrarily assumed that the standard error of v equals 20% and that of $M1$, 25% of the parameter value. In the extended stochastic sensitivity analysis, the resampling of the input parameters was repeated 500 times.

In addition, a retrospective analysis was performed to see the changes of the estimated biomass in 1982–1992 when some catch terms were omitted from the objective function. First, the model was fitted to the catch data from 1982 to 1991; next, the 1991–1992 catch data were omitted when fitting the model and so on, ending with fitting the model to the 1982–1986 catch data.

Results and discussion

The estimated parameters $\hat{G}$, $\hat{q}$, $\hat{b}$, $\widehat{B0}$, and $\widehat{\mathrm{OT}}$ and the bootstrap estimates of their standard errors are presented in Table 2. Most of the CVs are less than 20%. The CV of b for cod is very large. In initial bootstrap runs b was estimated to be a negative value. Therefore, for the final bootstrap procedure a lower constraint of 0.000 001 was imposed on b for cod. In 70% of the bootstrap replicates, b was estimated at its lower constraint. The suitability $G_{\mathrm{c}}^{\mathrm{hr}}$ is comparable with the suitability determined using the analytical formula from Horbowy (1989)

Table 2. Estimates, standard errors, and coefficients of variation for the parameters estimated by the multispecies production model.

Parameter	Estimate	SE	CV(%)
$G_{\mathrm{c}}^{\mathrm{sp}}$	1	—	—
$G_{\mathrm{c}}^{\mathrm{hr}}$	0.367	0.094	25
q_{c}	0.336	0.026	8
q_{hr}	0.877	0.165	19
q_{sp}	0.678	0.120	18
b_{c}	0.0158	0.032	206
b_{hr}	0.0188	0.0019	10
b_{sp}	0.0027	0.0004	15
$B0_{\mathrm{c}}$	944	55	6
$B0_{\mathrm{hr}}$	1544	201	13
$B0_{\mathrm{sp}}$	673	70	10
OT	3626	471	13

Note: See text for a description of the parameters. The $B0$s and OT are in thousands of tonnes. c, cod; hr, herring; sp, sprat.

$$G_{\mathrm{c}}^{\mathrm{hr}} = \left(0.22 \tan \mathrm{h} \left(\frac{B_{\mathrm{hr}}}{B_{\mathrm{sp}}} - 4.4\right) + 0.72\right) \times \exp\left(\frac{-(\ln(w_{\mathrm{c}}/w_{\mathrm{hr}}) - 4.15)^2}{1.16}\right),$$

where tan h is hyperbolic tangent function. Putting mean weight of cod and herring and mean biomass of herring and sprat in 1982–1992 into the formula produces $G_{\mathrm{c}}^{\mathrm{hr}}$ equal 0.29.

The majority of the estimated parameters are not highly correlated with each other (Table 3). Highly correlated are parameters q_{c} and b_{c}, q_{sp} and b_{sp}, b_{c} and $B0_{\mathrm{c}}$, and $G_{\mathrm{c}}^{\mathrm{hr}}$ and OT (correlation coefficient equalling 0.97, –0.75, –0.73, and 0.73, respectively).

The catch residuals for cod, herring, and sprat are presented in Fig. 1. Most of them are in the range from –0.20 to 0.20, which is equivalent to the relative error of modelled catches in the range of –18% to 22%. The sprat residuals for 1988 and 1989 are very high and these were excluded when fitting the final model. The residual distribution suggests that the 1988 and 1989 sprat residuals were outliers; their values were outside the interval defined as: lower quartile – 1.5(interquartile range), upper quartile + 1.5(interquartile range). The relative error of the modelled quotient of the consumed herring biomass to the consumed sprat biomass equals –22% for 1982 and 22% for 1987.

The estimated biomass of cod in the model decreased from 944 000 t in 1982 to 164 000 t in 1992 while the bio-

Table 3. Coefficients of correlation among parameters *q*, *b*, *G*, *B*0, and OT determined by the bootstrap procedure.

Parameter	q_{hr}	q_{sp}	b_c	b_{hr}	b_{sp}	$B0_c$	$B0_{hr}$	$B0_{sp}$	G_c^{hr}	OT
q_c			0.97	–0.23	–0.21	–0.59			–0.18	
q_{hr}		0.32		–0.31	–0.34		–0.50	–0.25	0.40	
q_{sp}				–0.36	–0.75	–0.28	–0.24	–0.36	–0.28	–0.46
b_c				–0.24	–0.25	–0.73				
b_{hr}					0.31	0.30			0.29	0.20
b_{sp}						0.41	0.25			
$B0_c$							0.25	0.19		
$B0_{hr}$									–0.34	0.18
$B0_{sp}$										0.36
G_c^{hr}										0.73

Note: Only significant correlations are shown. c, cod; hr, herring; sp, sprat.

Fig. 1. Log catch residuals of cod, herring, and sprat in the final multispecies production model fit.

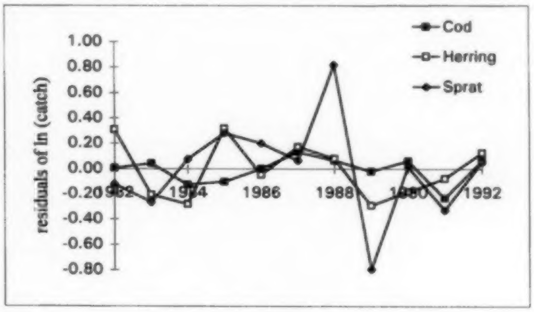
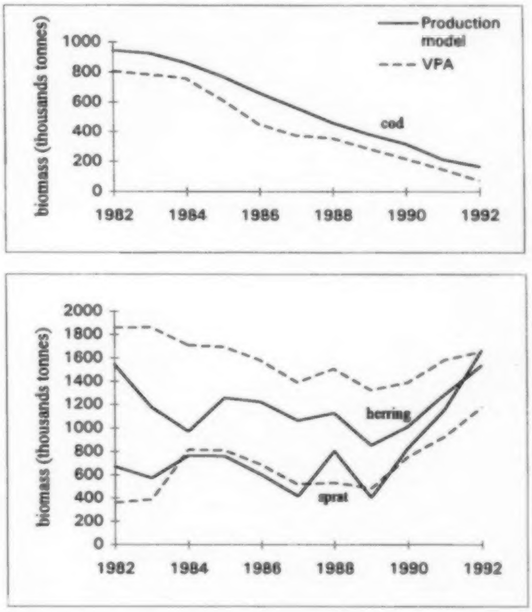


Fig. 2. A comparison of the biomass of cod, herring, and sprat estimated from the multispecies production model, with corresponding estimates obtained from virtual population analysis (VPA) (Anonymous 1994*a*, 1994*b*) for 1982–1992.



mass of herring was rather stable ranging from 900 000 t to 1 500 000 t. The biomass of sprat exhibits greater variation than herring biomass, varying from about 400 000 t in 1989 to about 1 700 000 t in 1992 (Fig. 2).

In Fig. 2, biomass estimates based on VPA and performed by ICES working groups (1994a, 1994*b*) are also presented for comparison. In Anonymous (1994*b*), natural mortalities used to run VPA are taken from MSVPA results, so the multispecies interactions are taken into account when assessing herring and sprat stocks. The production model values of cod biomass are similar to the working groups' estimates, but the biomass of cod estimated in the production model is consistently higher. The production model estimates of cod biomass are consistent also with those of Horbowy and Netzel (1992), based on the CAGEAN model (Deriso et. al. 1985). The estimates of the herring biomass are lower by about 25%, on average, in the production model relative to the VPA estimates. The sprat stock in the production model refers to fish in the waters east of Bornholm, while the Anonymous (1994*b*) estimates refer to the sprat from the whole Baltic. However, the largest amount of Baltic sprat inhabits Subdivisions 26 and 28 and usually less than 10% of sprat are caught in waters west of Bornholm. Thus, the sprat biomass in the production model is only slightly higher than the estimate obtained by the working group when the difference in the stock's distribution is taken into account.

The estimated predation mortalities of herring and sprat (Table 4) have systematically declined during the analyzed period (from 0.3 and 0.9 to 0.04 and 0.12 for herring and sprat, respectively) reflecting the decline in the biomass of cod. The predation mortality of both herring and sprat in 1992 is only about 13% of the estimate for 1982. The rate of decline in the predation mortality is similar to the rate of decline in the biomass of cod.

The estimated biomass of herring and sprat consumed by cod (Table 4) decreased from 740 000 t in 1982 to about

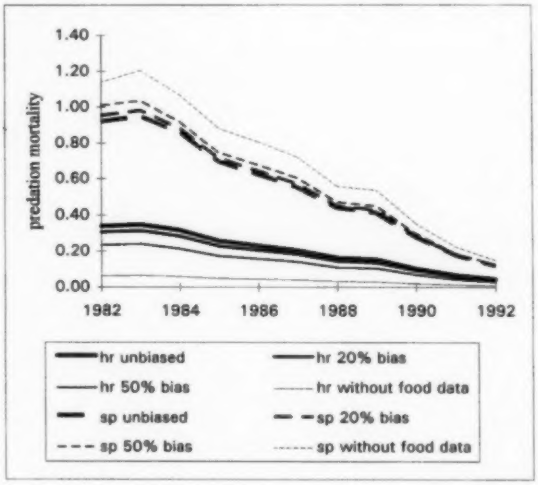
Table 4. Predation mortality and consumed biomass (thousands of tonnes) of herring and sprat calculated according to the multispecies production model (age 2 and older) and consumed biomass determined by Horbowy (1989) and in Anonymous (1994*c*) (age 0 and older).

			Consumed biomass					
	Predation morality		Production model		Horbowy (1989)		Anonymous (1994*c*)	
Year	Herring	Sprat	Herring	Sprat	Herring	Sprat	Herring	Sprat
1982	0.34	0.92	374	365	216	543	441	373
1983	0.35	0.95	302	325	219	514	401	524
1984	0.32	0.86	231	431	215	358	362	373
1985	0.26	0.70	243	361			254	235
1986	0.23	0.62	230	256			178	146
1987	0.20	0.55	169	152			150	143
1988	0.16	0.44	145	251			144	136
1989	0.15	0.41	106	124			120	134
1990	0.10	0.28	91	186			89	115
1991	0.07	0.18	79	176			37	86
1992	0.04	0.12	66	175			24	60

240 000 t in 1992. The multispecies interactions in the central Baltic during last years were rather intensively assessed (Horbowy 1989; Bundgaard and Sparholt 1992; Jensen and Sparholt 1992; Anonymous 1994*c*). However, it is not easy to make a direct comparison of the results obtained as the time span, area of application, and range of ages varied in the various approaches. In Table 4, the consumption by cod estimated in the multispecies production model is compared with cod consumption estimated by the ICES working group on multispecies assessment of Baltic fish (Anonymous 1994*c*) and Horbowy (1989). The estimates by Jensen and Sparholt (1992) are very similar to the Anonymous (1994*c*) values. When comparing these estimates, it should be borne in mind that the present model uses populations of mature fish (age 3+ for cod, age 2+ for herring and sprat), while the other estimates also cover age-groups 0 and 1. Taking this into account, it may be concluded that the various estimates of the biomass consumed by cod are rather consistent. However, the rate of decline of consumption in the production model is lower than that in Anonymous (1994*c*). When attempting to compare the obtained coefficient of natural mortality due to predation with the estimates from other sources, it should be remembered that in the model presented, $M2$ may be interpreted as an arithmetic mean of natural mortality coefficients in age-groups, weighted by the biomass of these age-groups.

When applying the multispecies production model, it was assumed that the term in brackets in eq. 1 (i.e., $a(t)$ in eq. 2) was constant in time interval $(t, t + \mathrm{d}t)$, and $\mathrm{d}t$ was set to 1 year. To check the sensitivity of the model to the assumed value of $\mathrm{d}t$, some simulations were conducted with $\mathrm{d}t$ equal to 0.5 and 0.25 year. The results were very similar to those obtained when $\mathrm{d}t$ was equal to 1 year. The $a(t)$ values are relatively independent of the change in mean weight during the time interval $(t, t + \mathrm{d}t)$, as a change of $\bar{w}$ by $p\%$ results in a change of $\bar{w}^{-1/3}$ by less than $p/3\%$. However, $a(t)$ is also dependent on biomass, so the time interval in which $a(t)$ is assumed constant should be determined by testing the model with different values of $\mathrm{d}t$.

Fig. 3. Multispecies production model estimates of the predation mortality of herring and sprat when food terms are excluded from the objective function or food terms are unbiased, biased by 20%, and biased by 50%. hr, herring; sp, sprat.



The production model was also run with the sprat outliers included, to see their influence on the estimates. On average, the inclusion of outliers resulted in 15% higher sprat biomass, 5% lower cod biomass, and almost identical herring biomass. The predation mortality of sprat changed remarkably, being lower than that estimated in the basic run by about 20%, and the predation mortality of herring changed only slightly.

When the production model is fitted without the food terms in the minimized objective function, the estimates of biomass for herring and sprat stocks are very similar to the ones obtained from the model with food terms included. However, the estimated suitability of herring was only 0.05, i.e., seven times lower than in the basic model. As a result, predation mortality of herring (Fig. 3) was much lower than in the basic model, and predation mortality of sprat was higher, leading to a much lower estimated ratio of herring biomass to sprat biomass consumed by cod. A decrease in the suitability of herring was compensated by a decrease in recruitment (smaller parameter *b*). Similarly, an increase in the predation mortality of sprat was compensated by an increase in its recruitment.

Next, the model was fitted with a biased input ratio of herring biomass to sprat biomass consumed by cod. The bias of this value by 20% had little influence on the predation mortality (Fig. 3). A more pronounced change in predation mortality is seen when the ratio is biased by 50%: the mortality of herring decreased by about 30% and the mortality of sprat increased by about 8%.

To check the influence of the multispecies interactions on the estimated population dynamics, the production model was also run for each species separately with no multispecies effects. In these runs, the natural mortality of herring and sprat was assumed to be equal to a mean of the predation

Fig. 4. Estimates of herring and sprat biomass obtained with the final multispecies production model fit (Mult.) and biomass estimates from the same model with total natural mortality kept constant in 1982–1992 (Single, i.e., multispecies interactions excluded from the model.)

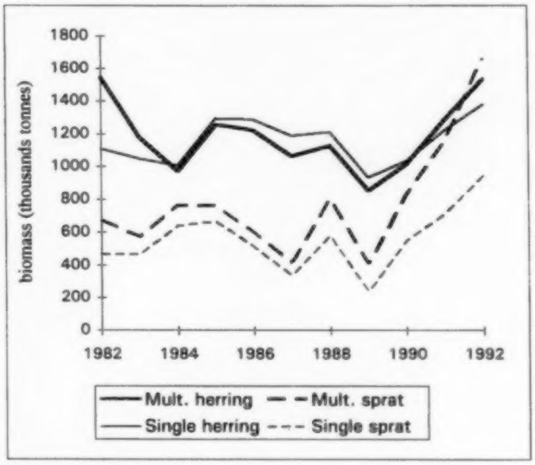
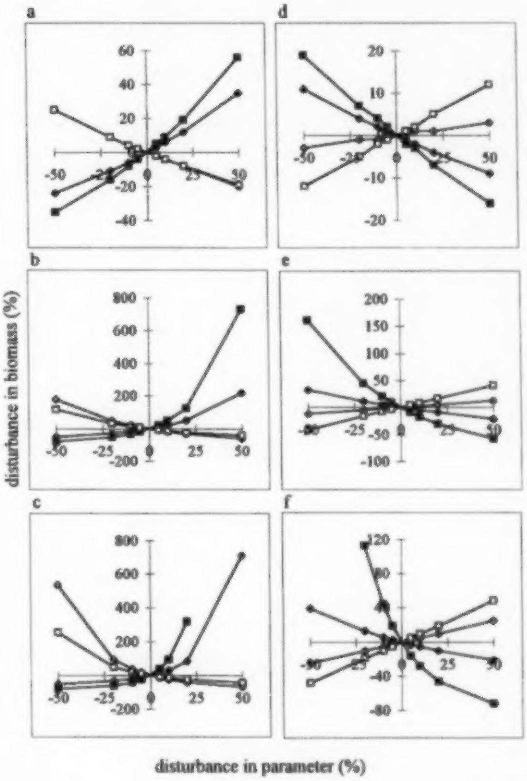


Fig. 5. Selected sensitivity analysis results for cod and herring: the disturbance of the biomass due to disturbance of the parameter. In Figs. 5*a*, 5*b*, and 5*c*, the influence of errors of h and k (■, h_c; ◆, h_{hr}; □, k_c; ◇, k_{hr}) on biomass in 1983, 1987, and 1992 is shown, while in Figs. 5*d*, 5*e*, and 5*f*, the same is shown for q and b (■, q_c; ◆, q_{hr}; □, b_c; ◇, b_{hr}).



mortality values estimated for 1982–1992 in a multispecies approach and enlarged by the residual natural mortality. The estimates of cod biomass are very similar in both models. The multispecies estimates of herring biomass are higher at the beginning and at the end of the 1982–1992 period and are slightly lower in the second half of the 1980s (Fig. 4). The multispecies estimates of sprat biomass are higher than the single-species estimates, and this difference increases in most recent years. The variation of biomass in the multispecies model is larger for herring (2.4 times) and for sprat (3.6 times) than in the single-species model. The sum of the residual catch terms for sprat in the objective function is almost four times lower in the fitted multispecies model than in the single species approach, while these terms for cod and herring are very similar in both approaches. It can be expected that the multispecies model will produce a better fit to the data than the single-species model, as shown by the fit to the sprat catches, which is much better in the multispecies model, indicating that predation by cod should be included when estimating the dynamics of sprat stock. The inclusion of the initial biomass parameter in the minimized objective function appeares to be crucial in case of the single-species model. Without it, the model estimates of sprat biomass were very low (in the range of 60 000 – 400 000 t), and the herring biomass was unrealistically high, ranging between 50 and 60 million tons. In the multispecies model, the exclusion of auxiliary information on initial biomass resulted in fitting a rather high herring biomass (2–3 million t), while cod and sprat biomass values were higher than in the basic run by about 10 and 20%, respectively.

Next, the model was run with the constant mean weight of fish in the populations. Two runs were made: one with a mean weight equal to the 1982–1992 grand mean of mean weights, and the other with a mean weight assumed equal to the 1982 level. The obtained biomass estimates differed from the values arrived at in the basic run by 5–13% on average, and the largest difference was 20%. This suggests that when the mean weight data series is not available, it practically can be replaced with the value from a selected year.

The ordinary sensitivity analysis of the model reveals that the accuracy of the growth parameter, v, h, and k has the greatest influence on the model results (Fig. 5). The disturbance in the values of these parameters results in a change of the biomass in 1983 at a level usually smaller than the level of disturbance. In 1987, the change in biomass is most often higher than the value of disturbance of v or h, and in 1992 the change in biomass may be even several times higher than the level of disturbance of these two growth parameters. The model is less sensitive to errors in parameter k. When the disturbance increases the value of the parameter, the change in the modelled biomass is usually lower than the value of the disturbance, while at a lowered value of k, the change in the biomass may be very high. Errors in parameters v, h, and k for cod also influence the modelled biomasses of herring and sprat; these errors usually are not higher than the errors of the parameters. Disturbances in the remaining parameters of the model (Fig. 5) cause changes in the calculated biomass at a level usually not exceeding the disturbance of the parameter even in 1992.

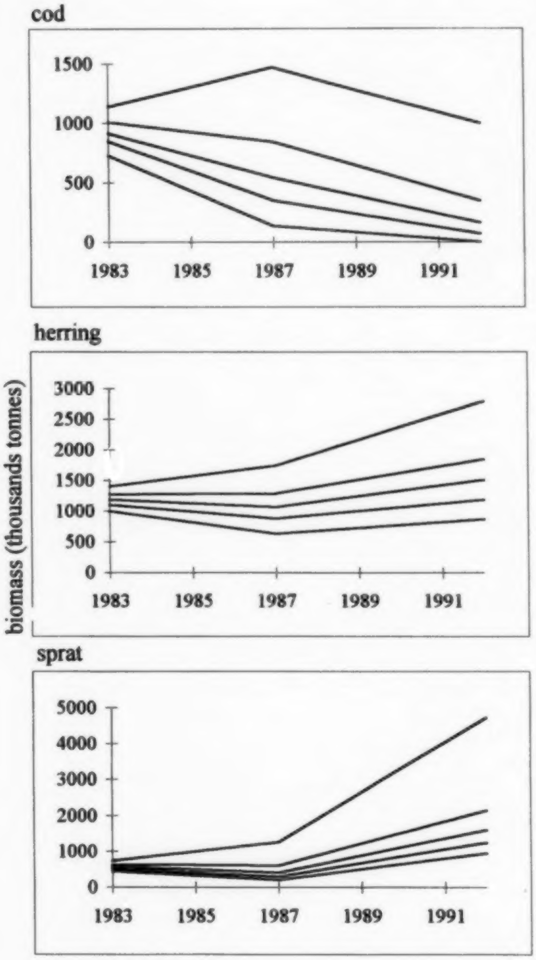
These sensitivity analyses show that the model is most sensitive to the change in growth parameters. This should not be a serious problem in the application of the model as the parameters H and k of the von Bertalanffy growth equation are easy to determine when weight-at-age data are available. However, for the predator in the model, one has to separate v and h from H. A biased v will produce a biased h, being the proportionality coefficient in the term expressing predation mortality in eq. 1. The v value used in the present simulations is similar to the v resulting from the Jobling (1982) formula. In this formula, developed for the North Sea cod, $v = 0.688w^{-0.156}$, which produces v values ranging from 0.25 to 0.20 for ages 3 to 9 for Baltic cod.

The results of the extended stochastic sensitivity analysis of the multispecies production model are presented in Fig. 6 as percentiles of biomass distribution. Distributions of the resulting biomass are skewed, and the skewness increases with time. The skewness of the distributions results from the nonlinear nature of eq. 2 and the assumed normal distributions of errors of the model parameters. For all three species, the upper and lower quartiles of the disturbed biomass distribution in 1983 differ by less than 10% from the median biomass. The quartiles of the biomass distribution in 1987 differ by about 20% from the median for herring, and by 30–60% for cod and sprat. The quartiles of the 1992 biomass distribution differ from the median by 20–30% for herring and sprat, and by 60–110% for cod. Thus, herring biomass was most resistant to parameter perturbations. The high response of cod biomass in 1992 to errors in input parameters is partly connected with a very low biomass of cod when compared with the beginning of the simulation period; estimation of the relatively smaller quantities may produce relatively higher errors.

Results of the retrospective analysis (Fig. 7) show that omitting the most recent catch data when fitting the multispecies production model has little influence on the biomass estimates. Omitting the catch data from the next 3–4 years leads to a change in biomass estimates of 30% at most, when compared with the run based on the 1982–1992 catch data. For all three species, fits of the model based on a smaller set of catch data result in lower estimates of biomass than those obtained with the model fitted to the catches during 1982–1992.

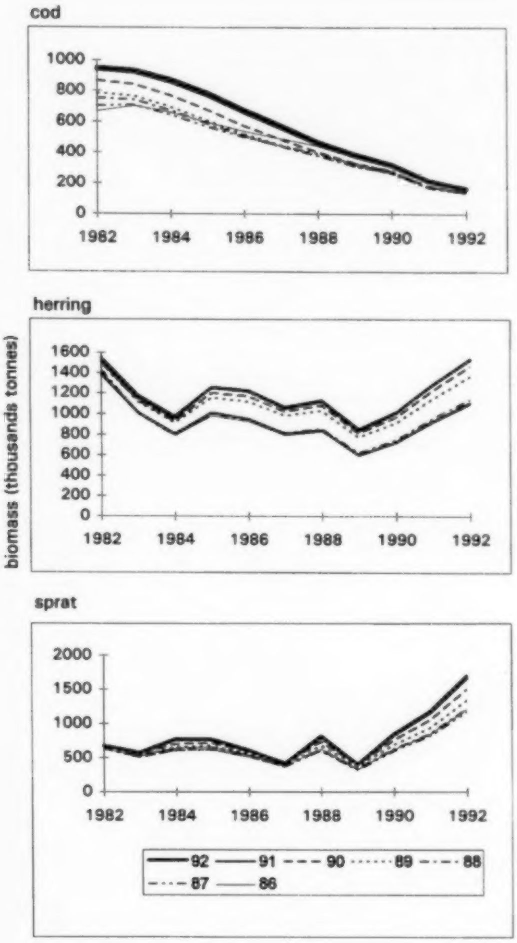
Recruitment constitutes an important part of the model, and the recruitment data should be reliable. In the case of the Baltic stocks, the data on the recruitment of herring are of rather poor quality. In the model, the results of the Polish young fish survey in the Gulf of Gdańsk were used, although they do not correlate well with the VPA estimates of year-class strength. The other possibility was to apply acoustic estimates of abundance of young herring. However, these estimates produced a worse fit of the model than those from the Polish survey on young fish. This may be explained by the distribution of young herring: they occupy shallower waters, which are only partly covered by the acoustic survey. Another alternative would be to apply a stock–recruitment relationship to generate recruitment in the model. However, such a relationship for the analyzed Baltic stocks has not been established.

Fig. 6. Extended stochastic sensitivity analysis results. The 5, 25, 50, 75, and 95 percentiles of the biomass estimate distributions for cod, herring, and sprat in 1983, 1987, and 1992 are shown.



As it has been already mentioned in the Introduction, the most popular and best tested multispecies model is probably the MSVPA. To run the MSVPA, one needs to know catch-at-age in numbers, stomach content data, and the estimates of the food ration of predators. The data are usually compiled by quarters, as some predators change their feeding behavior throughout the year. Other food is usually assumed constant. The crucial problem of MSVPA is the selection of terminal fishing mortalities. This could be circumvented by a procedure in which the estimated quantities are tuned to the observed values (e.g., catch per unit of effort, biomass estimates from the survey, indices of recruit numbers). However, such a procedure has not been implemented yet. The advantage of the presented multispecies production model is that it does not require knowledge of the age structure of the populations. On the other hand, the MSVPA does not require

Fig. 7. Retrospective analysis results for the biomass estimates when the catch terms for 1992, 1991–1992, 1990–1992, 1989–1992, 1988–1992, 1987–1992, and 1986–1992 were excluded from the objective function used to fit the model. For comparison, the biomass estimates based on all catch terms from 1982 to 1992 included in the objective function are presented.



recruitment indices and can, when tuned, provide historical estimates of recruitment.

The Schaefer multispecies model (Larkin 1966; Pope 1976) is less data demanding than the present model. To be fitted, it requires catch and effort data only. However, the number of parameters to be estimated within the model is five per species (including the initial biomass estimate), and it is questionable whether there is enough information in the catch and effort data to fit the model. The advantage of the present model is that some of its parameters can (and should) be estimated independently of the model (e.g., growth parameters) which reduces a number of parameters to be estimated within the model to three or four per species.

Conclusions

(1) The analyses show that the multispecies stock–production model can estimate the dynamics of the cod, herring, and sprat in the Baltic, producing results that are consistent with those of age-structured models.

(2) The comparison of the multispecies production model results with a single species approach suggests that predation by cod should be taken into account when estimating the dynamics of sprat stock.

(3) The retrospective analysis shows that, for these data, the multispecies production model produces relatively consistent results when some catch terms are excluded from the objective function.

(4) The exclusion of the food terms from the objective function results in erroneous estimates of the trophic interactions. However, a moderate bias in the data on food composition has a relatively small effect on the model estimates of both biomass and predation mortality.

(5) The model is most sensitive to the change in growth parameters.

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Appendix

We will present the derivation of eq. 1, starting from basic relationships of the Andersen and Ursin model (1977). In the Andersen and Ursin model, changes in abundance, N, mean weight, w, and cumulated catch, Y, are described by the following set of differential equations:

$$\begin{aligned} &\frac{\mathrm{d}N_{as}}{\mathrm{d}t} = -(F_{as} + M1_{as} + M2_{as})N_{as} \\ \text{(A1)}\quad &\frac{\mathrm{d}w_{as}}{\mathrm{d}t} = v_s h_s f_{as} w_{as}^{2/3} - k_s w_{as} \\ &\frac{\mathrm{d}Y_{as}}{\mathrm{d}t} = F_{as} N_{as} w_{as} \end{aligned}$$

where t is time; F is fishing mortality coefficient; $M2$ is the coefficient of natural mortality due to predation; $M1$ is the coefficient of natural mortality due to reasons other than predation; f is the indicator of the size of consumed food ration; v, h, and k are growth equation parameters, a is the age-group; and s is the species (population). The values of $M2$ and f are modelled with the following equations:

$$f_{as} = \frac{P_{as}}{P_{as} + Q_{as}}$$

$$M2_{as} = \sum_{r=1}^{n} \sum_{b=1}^{m_r} h_r \, \frac{G_{br}^{as} N_{br} w_{br}^{2/3} f_{br}}{P_{br}}$$

$$P_{as} = \sum_{r=1}^{n} \sum_{b=1}^{m_r} G_{as}^{br} N_{br} w_{br}$$

where P is available food, Q is the parameter defining food demands of the population, G_{br}^{as} is the indicator of preference as food of age group a of population s by age group b of population r, n is the number of populations considered, and m_r is the number of age-groups in population r.

Let B_{as} denote biomass of age group a of population s. Then

$$B_{as} = N_{as} w_{as}$$

Differentiating the above equation we obtain

$$\text{(A2)}\quad \frac{\mathrm{d}B_{as}}{\mathrm{d}t} = \left(\frac{\mathrm{d}N_{as}}{\mathrm{d}t}\right) w_{as} + N_{as}\left(\frac{\mathrm{d}w_{as}}{\mathrm{d}t}\right)$$

Next, we will assume that feeding level f is constant equalling 1. Substituting eq. A1 into eq. A2 we have

$$\frac{\mathrm{d}B_{as}}{\mathrm{d}t} = -\left(F_{as} + M1_{as} + \sum_{r=1}^{n}\sum_{b=1}^{m_r}\frac{h_r G_{br}^{as} N_{br} w_{br}^{2/3}}{P_{br}}\right) N_{as} w_{as} + (v_s h_s w_{as}^{2/3} - k_s w_{as}) N_{as}$$

$$= v_s h_s N_{as} w_{as}^{2/3} - \left(F_{as} + M1_{as} + k_s + \sum_{r=1}^{n}\sum_{b=1}^{m_r}\frac{h_r G_{br}^{as} N_{br} w_{br}^{2/3}}{P_{br}}\right) B_{as}$$

Adding the above equations according to age groups within the population and replacing magnitudes G_{br}^{as} and P_{br} with adequate averaged values for the populations, G_r^s and P_r and assuming that F and $M1$ are not age dependent, we obtain

$$\frac{\mathrm{d}B_s}{\mathrm{d}t} = \sum_{a=1}^{m_s}\frac{\mathrm{d}B_{as}}{\mathrm{d}t} = v_s h_s \sum_{a=1}^{m_s} N_{as} w_{as}^{2/3} - \sum_{a=1}^{m_s}\left(F_s + M1_s + k_s + \sum_{r=1}^{n}\sum_{b=1}^{m_r} h_r \frac{N_{br} w_{br}^{2/3} G_r^s}{P_r}\right) B_{as}$$

$$= v_s h_s \sum_{a=1}^{m_s} N_{as} w_{as}^{2/3} - \left(F_s + M1_s + k_s + \sum_{r=1}^{n} h_r \left(\frac{G_r^s}{P_r}\right) \sum_{b=1}^{m_r} N_{br} w_{br}^{2/3}\right) B_s$$

Using the following identity

$$\sum_{a=1}^{m_s} N_{as} w_{as}^{2/3} = \left(\frac{\bar{w}_s^{2/3}}{\bar{w}_s}\right) B_s$$

resulting from the definitions of the means $\bar{w}^{2/3}$ and $\bar{w}$ (Horbowy 1992*a*), we arrive at

$$\frac{\mathrm{d}B_s}{\mathrm{d}t} = v_s h_s \left(\frac{\bar{w}_s^{2/3}}{\bar{w}_s}\right) B_s - \left[F_s + M1_s + k_s + \sum_{r=1}^{n} h_r \frac{G_r^s}{P_r}\left(\frac{\bar{w}_r^{2/3}}{\bar{w}_r}\right) B_r\right] B_s$$

Presenting mean available food resources for the population and fishing mortality as

$$P_r = \sum_{i=1}^{n} G_r^i B_i + \mathrm{OT}$$

and

$$F_s = q_s E_s$$

we will finally obtain

$$\frac{\mathrm{d}B_s}{\mathrm{d}t} = \left[v_s h_s \left(\frac{\bar{w}_s^{2/3}}{\bar{w}_s}\right) - q_s E_s - M1_s - k_s - \sum_{r=1}^{n} \frac{h_r (\bar{w}_r^{2/3}/\bar{w}_r) G_r^s B_r}{\sum_{i=1}^{n} G_r^i B_i + \mathrm{OT}}\right] B_s$$

In practice, the ratio $\bar{w}_s^{2/3}/\bar{w}_s$ may be replaced by $(\bar{w})^{-1/3}$ (Horbowy 1992*a*) leading to a more compressed form of the model

$$\text{(A3)} \qquad \mathrm{d}B_s/\mathrm{d}t = \left(v_s h_s \bar{w}_s^{-1/3} - q_s E_s - M1_s - k_s - \sum_{r=1}^{n} \frac{h_r \bar{w}_r^{-1/3} G_r^s B_r}{\sum_{i=1}^{n} G_r^i B_i + \mathrm{OT}}\right) B_s$$

Analysis of vessel movements and strategies using commercial catch and effort data from the New Zealand hoki fishery

Marianne Vignaux

Abstract: Standardized catch rates from a multiplicative model of commercial catch per unit effort data have been examined to investigate vessel movements and strategies. A linear model was fitted to log-transformed commercial catch per unit effort data in the spawning hoki trawl fishery off the west coast of the South Island of New Zealand. The residuals from this model were examined; catch rates from tows done by different vessels at different times of day are standardized and, therefore, comparable. There is evidence that vessels make decisions about where to fish based on both their own recent catch history and on observations about the location and aggregation of other vessels. There is no evidence that there is enough information transfer for vessels to make decisions on the basis of catch rates of the other vessels in the fleet.

Résumé : On a examiné des valeurs normalisées de taux de capture obtenues au moyen d'un modèle multiplicatif des prises de pêche commerciale par unité d'effort dans le cadre de l'étude des déplacements et des stratégies d'un bateau de pêche. On a fait une transformation logarithmique des prises par unité d'effort effectuées dans une pêche commerciale au chalut du hoki en fraye, au large de la côte occidentale de l'île South, en Nouvelle-Zélande; on a ensuite ajusté un modèle linéaire aux données transformées. Les valeurs résiduelles obtenues au moyen de ce modèle ont ensuite été examinées; les taux de capture résultant de traits de chalut effectués par différents bateaux à différents moments de la journée ont été normalisés, si bien qu'ils sont comparables. Les décisions relatives au lieu de pêche seraient fondées tant sur les antécédents de capture récents du bateau que sur les observations relatives au lieu de pêche et au regroupement des autres bateaux. Rien n'indique que le transfert d'information soit suffisant pour que les décisions soient fondées sur le taux de capture des autres bateaux de la flottille. [Traduit par la Rédaction]

Introduction

Fishery dynamics, the study of fisher behaviour, is an important component of fishery science that has generally received little attention (Hilborn 1985). Understanding fishing strategies, the decisions that fishers make in deciding when and where to fish, are important both as part of a general understanding of the fishery and in predicting how a fishery might respond to proposed management changes, such as effort or area restrictions.

The decisions that fishers make about where to fish on a particular day are particularly important for the interpretation of catch rates as indices of abundance. Standardized catch per unit effort (CPUE) analyses (Gavaris 1980) using multiplicative models have become widely used in assessing fish stocks. They are often used to obtain an index of relative abundance of a stock, although it is now widely appreciated that there are many reasons why CPUE need not change linearly with abundance, (Cooke 1985) and indeed when stock size has been measured independently, CPUE has usually turned out not to be an index of abundance, and in some cases to be dangerously misleading (Peterman and Steer 1981; Shardlow 1993; Butterworth 1980).

It is clear that one of the reasons that CPUE may not be an index of abundance is because fishing behaviour or appropriate fishing strategies may allow vessels to maintain catch rates while abundance is falling. It is important to understand the fishing process to understand the relationship between stock size and CPUE (Hilborn 1985).

Some authors have inferred that skipper skill and strategies are important components in differential fishing success because they have observed differences in catching abilities that were not explained by differences in vessel characteristics (Hilborn and Ledbetter 1985).In other fisheries, however, (Palsson and Durrenberger 1982) vessel size and effort explained almost all the variability in the catch by different vessels, and there was no evidence for a significant skipper experience effect. Abrahams and Healey (1990) found that although the most successful vessels in the British Columbia salmon troll fishery also tended to be the largest, there were also differences in mobility between successful and unsuccessful vessels, and that even fishing in the same areas, the more mobile vessels outperformed the less mobile vessels.

Other work has looked in a descriptive way at the cultures of different groups of fishers and has made predictions about the behaviour of fishers that are testable from quantitative data. For example several papers have pointed out that fishers are generally risk averse. Palsson and Durrenberger (1982) and Andersen (1972) both suggested that this was because they needed to maximize their catch rates relative to the rest of the fleet so as to maintain social standing. Cove (1973) pointed out that one particular low-risk strategy was to fish where others

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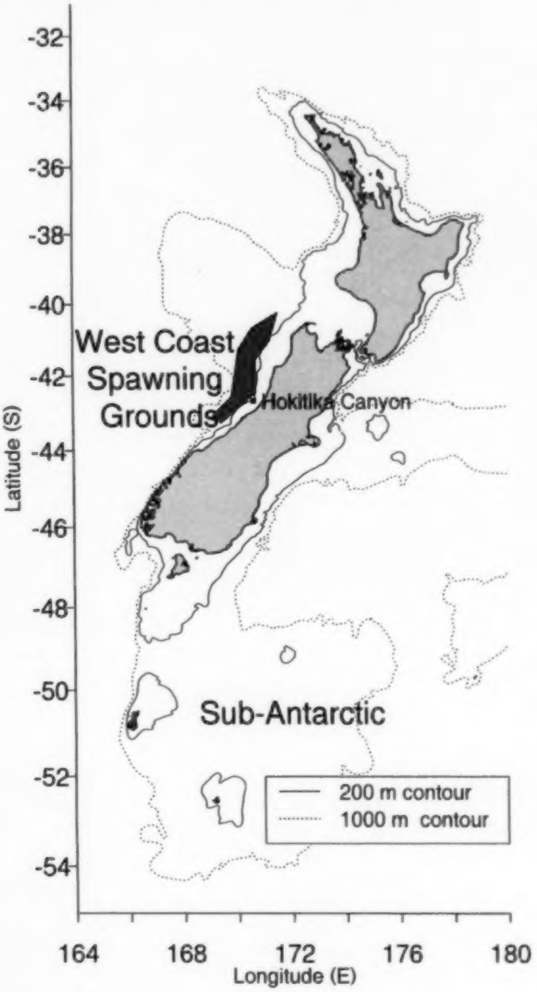
Fig. 1. Map showing the feeding grounds (sub-Antarctic) and the spawning grounds (west coast spawning grounds) of western stock of New Zealand hoki (*Macruronus novaezelandiae*).



fished. This can be justified in three ways. The presence of other vessels gives some information about the location of fish, which may be the most certain information available. Fishing with other vessels minimizes the risk that catch rates will be lower than average for the fleet. Furthermore, fishing with other vessels provides a rationale for a skipper's decision; even if catches are poor they are attributed to bad luck rather than a poor choice of location by the skipper.

Other work has made progress by proposing plausible fishing strategies and building models based on these strategies (Mangel and Clark 1983; Allen and McGlade 1986; Hilborn and Walters 1987; Sampson 1991), but do not test these models against quantitative data. Allen and McGlade (1986) described two different types of fisher: the "stochasts" who seek out new areas to fish, and the "cartesians" who go to the area with the best certain catch rate. They showed that the fishery could be most efficiently exploited if there was a mixture of these behaviours in the fishery.

Relatively few papers have looked at quantitative data and compared it with predictions from models of fishing strategies. Eales and Wilen (1986) found that fishers in the northern California pink shrimp fishery used recent landings information in a rational way in deciding where to fish. Hilborn and Ledbetter (1979) found that vessels in the British Columbia salmon purse-seine fleet took catch rate information from landings into consideration and also considered the relative desirability of different fishing areas. Gillis et al. (1993) found that vessels distributed themselves in a way that was consistent with a model of an ideal free distribution, in which CPUE was equalized among areas by the distribution of vessels.

This work extends this sort of approach by developing new techniques for looking at a complex commercial fishery: one in which there are a large number of vessels, with a range of catching abilities, where the fishing area is small so that vessels are making frequent decisions about where to fish, and where vessels do not land fish daily or even weekly, so that landings information cannot be used to transmit information about catch rates.

The data come from catch and effort reports from the commercial fleet. To obtain data that could be compared across vessels in the fishery, a multiplicative model similar to those often used to develop a CPUE index was first used to standardize the catch rates. Although there may be difficulties in using this data set to develop an index of abundance, the data set has proved to be useful for answering some of the initial questions, such as what information do the fishers use in deciding where to fish?

Background to the fishery

Hoki (*Macruronus novaezelandiae*) are a gadid species widely distributed in New Zealand waters. The largest stock, the western stock, is thought to have feeding grounds in the sub-Antarctic region south of New Zealand (Fig. 1), and to migrate to the west coast of the South Island to spawn in July and August each year (Sullivan et al. 1995).

There is an intense trawl fishery on the spawning aggregations, consisting mainly of large foreign vessels chartered by New Zealand companies. In 1993 there were 61 vessels in the fishery, in an area about 130 nautical miles long by 30 nautical miles wide (1 nautical mile = 1.852 km). The vessels range from 25 to 105 m overall length and come from a number of countries, including Japan, Korea, and Norway. They use midwater or bottom trawl nets in depths of 300–700 m. All vessels fishing in New Zealand waters are required to report both their catch and effort on forms submitted to the Ministry of Agriculture and Fisheries. Most of the larger vessels fill in detailed forms in which details about each tow are recorded.

The 1994 stock assessment estimated the biomass in 1995 of the western stock to be 1.54 million t, with a virgin biomass of 1.69 million t and a long-term average yield of about 185 000 t·year^{-1} (Sullivan et al. 1994).

Data standardization

Because catch and effort data comes from many different vessels fishing under different conditions, nearby tows are not

Table 1. Summary of variables in model.

Variable	Type[a]	Description
Year	Categorical (7)	Year that tow occurred in
Season	Continuous	Day relative to peak of season for that year
End time	Continuous	Time in decimal hours at end of tow
Latitude	Continuous	Position in decimal degrees at start of tow
Depth bottom	Continuous	Depth in metres of bottom at start of tow
Depth net	Continuous	Depth in metres of groundrope of net at start of tow
Net off bottom	Continuous	Distance between groundrope and bottom at start of tow
Headline height	Continuous	Headline height of net at start of tow
Vessel nation	Categorical (6)	Country of origin of the vessel
Vessel length	Continuous	Length overall of the vessel, in metres
Vessel tonnage	Continuous	Gross tonnage of the vessel
Vessel year built	Continuous	Year the vessel was built
Vessel power	Continuous	Power of vessel engines in kilowatts
Vessel breadth	Continuous	Breadth of the vessel, in metres
Vessel draft	Continuous	Draft of the vessel, in metres
Vessel processing	Categorical (4)	Processing category of vessel
Vessel lbd	Continuous	Vessel length × breadth × draft in cubic metres

[a] Continuous, continuous varible; categorical, category type variable with the number of categories given in parentheses.

directly comparable. Therefore, a multiplicative model was fitted to the CPUE data, attempting to remove as much of the variability due to vessel-vessel differences, seasonal, or time of day effects as possible. By using estimates of fishing power for these variables, which are based on catch rates from the entire data set, the variability that is due to other sources is highlighted. The residuals from one season's data from this model are the standardized catch rates that are discussed in the methods and results sections of this paper.

Data from the commercial fishery

All available tow by tow data for tows targetting hoki on the west coast spawning grounds between 1987 and 1993 were analysed. This area was defined as between longitudes 167°E and 172°E, north of latitude 46°S and between day 150–335 of the year (the beginning of June to the beginning of December). The target species was identified by the fisher.

For each tow, information as described in Table 1 was available. Information about the design of the net, such as bottom trawl or midwater net, was not available, but the headline height of the net and the distance of the net off the bottom may act as proxies. The length of the tow in nautical miles was estimated from the reported speed and duration of the tow.

Table 1 also indicates how each variable was modelled, either as a continuous variable, with up to three parameters (cubic), or as a categorical variable, with some given number of categories. Only direct effects were examined in this model. Individual vessel effects were not examined because of the large number of vessels involved in the fishery over the seven seasons.

The linear model

To build a multiplicative model of CPUE that explained as much as possible of the variation in CPUE a simple forward stepwise approach was taken (details of the model are described in Vignaux (1994) and the stepwise approach is described in Draper and Smith (1981)). The logarithm of catch per nautical mile was regressed against each of a set of variables in turn. The variable which explained the most of the variability in CPUE (provided the largest increase in R^2) was selected. This variable was then included in the model, and the CPUE regressed against it and each of the other predictor variables, to find the next most useful variable (Table 2). This stepwise regression procedure continued until no significant extra explaining power came from adding an extra variable to the model (R^2 improved by less than 0.5%). With more than 47 000 data points, an F-test to determine the significance of a variable was found to be inappropriate; all variables appeared to be significant, even when the effect of the variable was negligible. Therefore, the stronger requirement that a useful improvement in R^2 be observed was used. Variables that were modelled as polynomials were included or excluded as a group of three orthogonal vectors, with a reduction of 3 df.

Approximately 3% of the tows were reported as having zero catch. These were replaced with an arbitrary minimum catch of 1 kg before the logarithmic transform. Sensitivity tests showed that the results were not affected by the value of this arbitrary catch.

All modelling and analysis were done using the statistical programming environment S (Becker et al. 1988; Chambers and Hastie 1992).

Results of the linear modelling

The most significant variable is a seasonal effect, which is modelled as a cubic continuous variable. Since the season can be early or late in a particular year, depending on environmental factors, each season is expressed relative to the peak of the season for that year (estimated by fitting a smooth nonparametric curve through the raw catch rates for the year). The cubic polynomial shows an increase to a peak, followed by a relatively gradual decline.

The second most important variable is nationality,

Table 2. Summary of the stepwise regression.

	R^2 at iteration							
Variable	1	2	3	4	5	6	7	8
Season	10.23							
Vessel nation	4.52	16.30						
End time	1.69	11.83	18.25					
Year	2.22	12.09	18.14	20.14				
Vessel length	3.70	13.93	17.56	19.65	21.58			
Latitude	7.90	10.77	17.23	18.44	20.78	22.33		
Headline height	2.34	11.89	17.05	19.11	21.50	22.26	22.97	
Vessel year built	0.22	10.66	16.62	18.59	20.73	22.06	22.86	23.34
Vessel tonnage	2.99	13.18	17.56	19.59	21.48	22.01	22.77	23.19
Depth net	2.42	10.55	16.55	18.76	20.37	21.82	22.53	23.16
Vessel lbd	4.78	15.47	17.51	19.57	21.44	21.81	22.56	23.15
Vessel draft	3.98	15.08	17.24	19.27	21.16	21.79	22.55	23.14
Vessel processing	4.83	16.02	17.37	19.42	21.50	21.96	22.68	23.13
Depth bottom	1.14	11.04	16.84	18.56	20.29	21.72	22.48	23.12
Vessel power	3.77	14.40	17.48	19.53	21.45	21.75	22.50	23.09
Vessel breadth	4.22	14.57	17.49	19.58	21.54	21.66	22.44	23.07
Net off bottom	2.36	12.39	16.68	18.76	20.32	21.69	22.44	23.01
Improvement	10.23	6.07	1.95	1.89	1.44	0.75	0.64	0.37
df	47 329	47 323	47 320	47 313	47 310	47 308	47 305	

Note: In each iteration the variable that had the highest value of R^2 was included in the model. The iterations continued until the improvement in R^2 was less than 0.5%.

modelled as a categorical variable with a category for each of the different nations with vessels in the fleet (Japan, Korea, ex-Soviet, etc). This variable could reflect either design features in the vessels involved, different fishing gear, or different fishing practices.

The third most important variable is the time of day that the tow ended. Again, this variable is modelled with a continuous third-order polynomial, which increases through the day, peaks at about 15:00, and declines into the evening. This is consistent with fishers' perception of daily changes in catch rates in the fishery. Preliminary modelling suggested that the time at the end of the tow was a more useful variable than the time at the start of the tow, but either of these times, or indeed a time between the two could have been used.

The fourth most important variable is the year effect. This shows a systematic change in CPUE among years that cannot be explained by changes in the other factors and, therefore is interpreted as a change in abundance of the stock. Since it is not possible to determine what the vessels are doing between tows, it is not known to what extent vessels actively search for fish with echosounders, before beginning a shot. If searching was a dominant behaviour in the fishery, then catch rates may represent density within patches, rather than total abundance. This would affect the interpretation of the year effect. If this were the case we would not expect to detect strategic behaviour in the tow data, as vessels would be responding primarily to information that is inaccessible to any analysis.

The year effect obtained from the model is broadly similar to an index based on the geometric mean of CPUE or on the geometric mean of CPUE for those few vessels present throughout the fishery. Since there is little information available about the fish-finding technology on board the vessels, it is possible that the year effect is confounded with improvements in technology over the period of the data or with changes in strategy associated with the introduction of the new technology. Understanding such changes are part of the motivation for this work. However, the period 1987–1993 was chosen (based on anecdotal information) as a period of relative stability in the operation of the fishery.

Vessel size and the height of the net are minor variables (R^2 improved less than 1.5%), with the catching power of the vessel increasing with both size of vessel and size of net. Another minor variable models a trend in catch rates with latitude, with good catching areas in both the north and the south of the region. Again, these variables are modelled with cubic polynomials.

The final model can be summarized as follows (notation is as in Chambers and Hastie 1992): log(catch/nautical mile) ~ season + season^2 + season^3 + factor (nation) + time + time^2 + time^3 + factor(year) + length + length^2 + length^3 + latitude + latitude^2 + latitude^3 + headline + headline^2 + headline^3.

With seven direct-effect variables in the model the multiple R^2 is 23%, with 47 305 df. It is not surprising that only 23% of the variability can be explained using these crude variables, as even the best professional fishers have fluctuating catch rates within short periods. However, the model is necessarily incomplete because the information available is limited and prone to errors. In particular, there is no information on the technology available on different vessels, any details of the nets used, or the amount of searching that vessels do between shots.

The residuals from the 1993 data in this model are used as the data for the studies of vessel movement, since the standardization procedure has made tows done by different vessels at different times of day comparable. Standardization is necessary because the effects are significant; both statistically significant and because they have an important influence on the expected catch rates. There is a maximum factor of 3.0 difference in expected (raw) catch rates for vessels of different nationalities and a factor of 2.9 difference for tows done at different times within the 24-h period.

In the rest of this paper the residuals are referred to as standardized catch rates or simply catch rates. A single season of data was analysed because it is possible that strategies have changed over time. The 1993 season was chosen for closest examination because it was the most recent season in the data set. Preliminary examination of the data from the 1990 and 1992 seasons suggested, however, that similar patterns of vessel movement are also seen in those data sets. An alternative approach, if the seasonal and vessel catch rate patterns were very different from year to year, would have been to fit the data from the three seasons in three separate multiplicative models.

Although it is important to fit all factors in the model that are capable of explaining variance, including season, year, and latitude, it is not necessary to remove these effects from the standardized catch rates. Instead, the standardized catch rates could have been estimated using only some of the model parameter estimates, leaving variability due to the other variables in the residuals. It might actually be better not to detrend the catch rates with respect to latitude. Since the latitude has a minor effect on the standardization, it has been left in in this analysis.

Methods

For this study, the west coast fishery area was divided (on the basis of start positions) into 24 squares of side 30 nautical miles, aligned to the longitude line 167°E and the latitude line 39.6°S. Most tows are between 5 and 20 nautical miles long, but some are as long as 50 nautical miles. Since on average vessels move to a new square nearly every second day, a day was chosen as the unit of time. Using this spatial and temporal scale, approximately 40% of tows cross a spatial boundary and 8% cross a temporal boundary.

1. Vessel response to catch rate by altering effort

First, we determined whether vessels responded to catch rates by changing the amount of effort they allocated to an area. For each vessel, the average catch rate for that vessel over the season (using standardized catch rates) was used as a standard to identify fishing days with poor catch rates. The number of tows done and the mean catch rate of each vessel in each area on each fishing day was calculated. Therefore, the change in the number of tows in an area from one day to the next could be related to the mean catch rate in that area on that day, compared with that vessel's average catch rate over the season. The proportion of times that the number of tows stayed the same or increased was calculated for the two cases: the mean catch rate was above average, or the mean catch rate was lower than average. The changes in proportions were tested for significance using the normal approximation to the binomial distribution (this approximation was appropriate since there were more than 1000 vessel days).

The data were examined in this way to determine whether vessels responded to the value of their current catch rates relative to some measure of expected catch rates (approximated here by the mean value achieved over the season). The catch rates that other vessels are achieving at the same time (if known) may be another factor used in deciding where to fish or how to allocate effort. This question is examined later.

2. Vessel response to catch rate by moving away

Next, we determined whether when a vessel gets a poor catch rate, it moves further away before attempting another shot than when it gets a high catch rate. The distance from the end of each tow to the start of the next tow by that vessel was determined. Therefore, the distance the vessel moves can be related to the catch rate (again, using standardized catch rates).

3. Fleet response to catch rates by changing effort

Third, we determined whether the fleet as a whole responds to poor catch rates by putting less effort into that area. The number of tows done by the fleet in each area on each day was calculated. The mean standardized catch rate done by the fleet in that area was also calculated. Therefore, the change in the number of tows from one day to the next can be related to the mean catch rate.

4. Attraction to a new area

Fourth, we determined what factors attract a vessel that has moved away from an area where they were fishing into a new area. A set of potential strategies were proposed (Table 3), for example, "choose an area that had a higher than average mean catch rate yesterday." For each strategy, the number of occurrences of vessels behaving consistently with this strategy were examined and compared with the number that could be expected by chance alone. If the actual number of occurrences is significantly greater than the number expected by chance alone, then there is evidence that vessels are following the strategy. The details of the calculation of the actual and expected numbers of occurrences (and the standard error of the expected numbers) are given in the Appendix. In summary, the actual number of occurrences was determined by counting the number of times the vessel moved in a way that was consistent with the strategy being examined. The expected number of occurrences was determined by counting the number of areas that would have been consistent or inconsistent with the strategy and assuming that the vessel would (with no strategy) choose between the areas with uniform probability. The standard error of the expected number of occurrences was estimated using a bootstrap procedure.

5. Vessel crowding

Fifth, we determined whether the catch rate of a vessel is affected by the presence of other vessels. For each day, in each area, the newly arrived vessels were counted. The average catch rate (standardized) of vessels that had fished in that area on both the previous day and that day were evaluated for both days. Therefore, the change in catch rates for those vessels present on both days can be related to the number of new vessels that arrived.

6. Different strategies for different vessels

Sixth, we determined whether the factors that affect how a vessel decides where to fish are the same for all vessels, or whether there are groups of vessels that follow different strategies. To do this, we examined the results under the fourth objective on a vessel by vessel basis, to compare the actual number of times a vessel followed a strategy with the expected number if it had been behaving by chance alone. This gives a distribution of the ratios of actual to observed occurrences for the different vessels.

Then, to determine whether a distribution such as this could be obtained if the vessels were in fact all following the same general strategy, but random variation caused some vessels to have greater realized ratios than others, a simulation procedure was used (bootstrap procedures are described in Efron and Tibshirani 1993). This simulation was based on the idea that if all vessels were in fact following the same strategy, then it would be as if the movements for each vessel were selected randomly from the pool of all vessel movements.

So, for each vessel, each movement was selected randomly (with replacement) from the pool of 1799 observations of vessel

Table 3. Summary of potential strategies proposed for vessels that have moved into a new area which they did not fish on the previous day ("yesterday").

Strategy	Short name
1. Choose an area that had a higher than average mean catch rate yesterday	High mean
2. Choose an area that had a higher than average maximum catch rate yesterday	High maximum
3. Choose an area that had at least 25% of the fleet fishing there yesterday	Aggregation
4. Choose an area that was fished and that is adjacent to one of the areas you fished yesterday	Nearby
5. Choose an area that is adjacent to one of the areas you fished yesterday but that also had at least 25% of the feet fishing there yesterday	Nearby and aggregation
6. Given that you are going to an adjacent area, choose one that had at least 25% of the fleet there yesterday	Aggregation given nearby
7. Given that you are not going to an adjacent area, choose an area that had at least 25% of the fleet fishing there yesterday	Aggregation given not nearby
8. Follow a particular one of the 61 vessels in the fleet	Follow vessel *x*

Note: A short name is introduced for later reference.

movements. The actual and expected number of movements consistent with the strategy were then calculated separately for each of the 61 vessels. Each vessel will of course have the same expectation for the ratio of actual/expected movements (under the null hypothesis of uniform strategy), but random variation will cause different values of the ratio for each vessel, and in particular, vessels with few movements could have widely varying ratios.

The quantiles of the distribution of the ratios for the 61 vessels from this simulation were then examined and compared with the quantiles from the true distribution. The simulation was repeated 200 times.

Results

1. Vessel response to catch rate by altering effort

When a vessel's catch rate was high, it was more likely to stay on in an area or allocate more effort in it than if the catch rate was low. In cases where the mean catch rate was greater than the average for that vessel, 38.9% of the time the vessel did at least as many tows in the same area the next day, and 18.7% of the time it did more tows. When the mean catch rate was less than the average for that vessel, only 31.3% of the time did it do at least as many tows the next day, and 11.9% of the time it did more tows. This change in distribution is shown in Table 4.

The difference between 38.9% and 31.3% was found to have a t value of 4.8 and the difference between 18.7% and 11.9% has a t value of 5.7. The probability (p) of obtaining by chance t values at least as far from zero as these, under the null hypothesis that the distributions are the same (and with 925 df) is less than 1%. Both results are therefore significant at the 1% level.

2. Vessel response to catch rate by moving away

It seemed, therefore, that the vessels were following a sensible strategy with respect to the information they have about their own catch rates: if catch rates were high, they stayed in the area, and if catch rates were low, they moved out of the area.

If the catch rate is low in a particular tow, we might then expect to see the vessel moving further away than when the catch rate was high. Figure 2 shows cumulative distributions of distances travelled by vessels after tows with CPUE of less than or greater than their season average (the plot is for all vessels combined). When the catch rate was low, the distribution of distances travelled had more weight at larger distances than when the catch rate was high. The Kolmogorov–Smirnov test found these distributions to differ at the 1% level (with a p value of 2.6×10^{-10}).

Table 4. Proportions of times that a vessel put as much or more effort into an area after a mean catch rate below or above the average for the season.

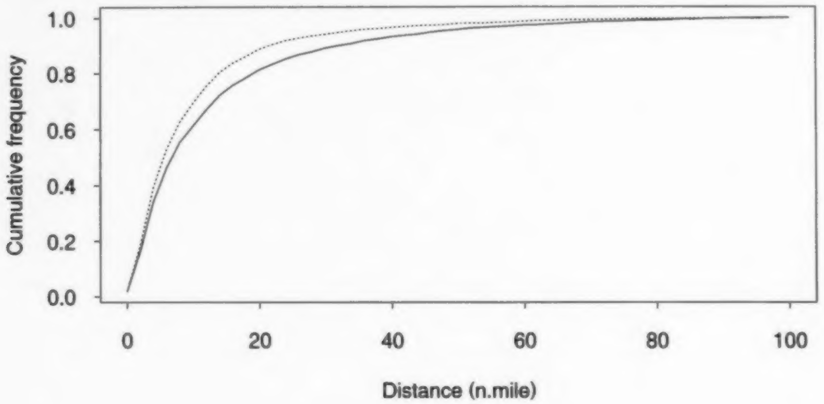
Catch rate	As much effort	More effort
CPUE > average (%)	38.9	18.7
CPUE > average (%)	31.3	11.9
t	4.8	5.7
p	1.9×10^{-6}	1.8×10^{-8}

When the catch rate was lower than the average for that vessel, 19.0% of the time the start of the next tow is more than 20 nautical miles away from the end of the tow. However, when catch rate is at least as high as the average, the next tow is more than 20 nautical miles away only 11.5% of the time. This difference in proportions has a t value of 8.66 and is significant at the 1% level (with 1635 df, p is negligible).

3. Fleet response to catch rates by changing effort

There was little evidence that vessels were responding to the mean catch rates of other vessels. If we do a similar calculation to the first calculation described above, and look at the mean catch rate over all vessels in an area on a day, we find that, when the mean catch rate was greater than average, 46.1% of the time there were at least as many tows done in the area the next day, and 30.6% of the time there was an increase in the number of tows (Table 5). If the mean catch rate was less than average, 43.4% of the time there were at least as many tows done in the area the next day, and 31.4% of the time there was an increase the next day. The difference between 46.1% and 43.4% has a t value of 0.69 and the difference between 30.6% and 31.4% has a t value of 0.23. The

Fig. 2. Cumulative distribution functions of the distances travelled by a vessel after a tow with a catch rate less than (solid line) or greater than (broken line) the season average for that vessel (all vessels combined).



probability (p) of obtaining by chance values at least as different as these under the null hypothesis that the distributions are the same (and with 168 df), is larger than 10%, so neither of these results are significant at the 10% level. There is, therefore, little evidence that vessels are responding to the mean catch rates of other vessels.

Table 5. Proportion of times that a fleet put as much or more effort into an area after a mean catch rate below or above the average for the season.

Catch rate	As much effort	More effort
CPUE > average (%)	46.1	30.6
CPUE > average (%)	43.4	31.4
t	0.69	0.23
p	0.49	0.82

4. Attraction to a new area

Vessels appeared to be attracted to an area when they saw a concentration of vessels already there.

To investigate the question "how do vessels that are new to the fishery, or that have decided to leave the area they are currently fishing, decide which area to try?", we looked for evidence that vessels were following one of a number of strategies.

Suppose that vessels have a strategy which is "choose an area that had a higher than average mean catch rate yesterday," assuming that there is relatively free exchange of information about catch rates, and vessels are able to deduce which areas these are.

Following the procedure outlined in the methods section and the Appendix, we find that on 844 occasions vessels moved to areas that would be consistent with this strategy (Table 6). By chance alone, however, we would have expected 849 movements consistent with this strategy. The standard error of the expected number of movements is 20, so the probability of obtaining a value at least as far from the expected value as was obtained (under the null hypothesis that the vessels were not following the strategy) is much more than 1%. Therefore, there is no evidence that vessels are successfully applying a strategy of moving towards areas where there was a high mean catch rate on the previous day.

If we consider the type of information the skippers may have about the catch rates of other vessels, we see that they are unlikely to have a good idea of the mean catch rate of the fleet. Perhaps the information that travels most quickly and is hardest to hide is information about very good catch rates.

Following the procedure outlined above, but using the strategy "choose an area that had a higher than average maximum catch rate yesterday," we see that on 995 occasions vessels moved to areas that would be consistent with this strategy, and by chance alone we would have expected 702 movements consistent with this strategy. With a standard error of the expected number of movements of 20, the probability of obtaining a value at least as far from the expected value as was obtained (under the null hypothesis that the vessels were not following the strategy) is in this case much less than 1%. This may indicate that vessels are attempting to apply this strategy.

The other piece of information vessels may have about what other vessels are doing, is their position, which may be visible on radar. If we propose a strategy "choose an area which had at least 25% of the fleet fishing there yesterday," then while 411 occurrences would be expected by chance, there were 764 actual occurrences. Again, with a standard error of 16, the probability of obtaining a value at least as far from the expected value as was obtained (under the null hypothesis) is extremely small (much less than 1%). The observed value is 22 units of the standard error away from the expected value, even more than under the "high maximum" strategy. This suggests that vessels are significantly more likely to move to an area in which there was an aggregation of vessels on the previous day.

Of course, vessels are unlikely to choose to fish in an area that is far away when there is a neighbouring area which seems promising. If we propose a strategy "choose an area that was fished and that is adjacent to one of the areas you fished yesterday," then while 713 occurrences would be

Table 6. Comparison of the actual number of occurrences of a movement of a vessel into a new area that was consistent with a given strategy, with the number of occurrences that would be expected if the vessels were moving by chance alone.

Strategy	Actual	Expected	Ratio	SE	Difference	*p* value
1. High mean	844	849	0.99	20	−0.2	0.84
2. High maximum	995	702	1.42	20	15	0
3. Aggregation	764	411	1.86	16	22	0
4. Nearby	1095	713	1.54	18	21	0
5. Nearby and aggregation	545	214	2.54	12	28	0
6. Aggregation given nearby	545	329	1.66	13	17	0
7. Aggregation given not nearby	219	152	1.44	9	7	3×10^{-12}
8. Follow vessel *x*			See Figure 3			

Note: The standard error of the expected number of occurrences and the ratio of the actual to the expected numbers are also given. This ratio is a measure of the size of the effect. The last two columns are the differrence between the actual and the expected number of occurrences measured in units of the standard error of the expected number, and the probability of obtaining a difference this large under the null hypothesis that the vessels were not using this strategy.

expected, there were 1095 actual occurrences (again, significant at the 1% level).

This suggests that a combination of two strategies might be appropriate: "choose an area that is adjacent to one of the areas you fished yesterday but that also had at least 25% of the fleet fishing there yesterday." In this case, there are 214 occurrences expected and 545 actual (again, significant at the 1% level).

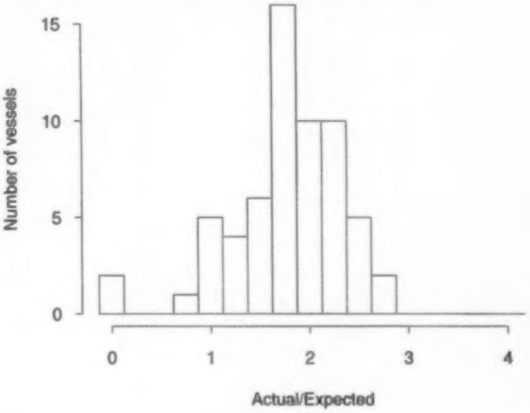
To determine whether vessels follow aggregations whether or not they are moving to a nearby area, the two situations were examined separately. Both the strategy "given that you are going to an adjacent area, choose one that had at least 25% of the fleet there yesterday" and the strategy "given that you are not going to an adjacent area, choose an area that had at least 25% of the fleet there yesterday," have significantly (at the 1% level) more actual occurrences than you would expect, particularly the former strategy. It may be that, when a vessel decides to move further away, it no longer has such accurate information about where vessels were fishing on the previous day.

It is also possible that vessels are not following the aggregation of vessels themselves but that some vessels are following particular other vessels in the fleet. To investigate this possibility, the strategy "move to an area where vessel *x* fished yesterday" was investigated for each of the 61 vessels in the fishery. As would be expected there was a consistent effect of most vessels having a positive attractive effect on the fleet. This is because the presence of a vessel in an area is correlated with the presence of an aggregation in that area.

The median ratio of actual/expected for the 61 particular examples of this strategy was 1.73. As shown in Fig. 3 the distribution of actual/expected does not suggest that there are two groups of vessels: those that are followed frequently and those that are followed rarely. The two vessels that were never followed were in the fishery for only 2 and 6 days, respectively.

There is a significant correlation ($p = 0.67$ with 59 df, significant at the 1% level) between the ratio of actual/expected for a vessel and the proportion of days that the vessel spent in aggregations containing at least 25% of the fleet. That is, the vessels that appear to be followed more often, are the ones that are more often in an aggregation of vessels. Therefore, the appearance of being followed may be an artifact.

Fig. 3. Histogram of 61 ratios of actual/expected occurrences of vessels moving to areas consistent with a strategy of following each of the 61 vessels in the fleet. The ratios are taken over the whole fleet, for each of the 61 different particular strategies.

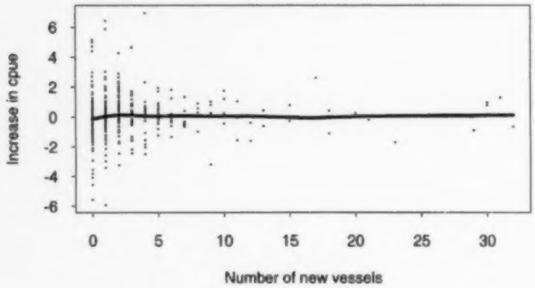


It seems then, that among other things, vessels appeared to be attracted to an area when they saw a concentration of vessels already there. The apparent relationship between the maximum catch rate and the number of vessels attracted may be due to a relationship between the number of vessels present and the maximum catch rate.

5. Vessel crowding

It has been suggested that a large number of vessels in an area on a day can depress catch rates by causing interference between vessels. If vessels are attracted to these areas anyway, maybe this effect is not great compared to the relative advantage of being in the high catch rate area.

Fig. 4. Relationship between the number of new vessels that arrive in an area on a day and the change in mean catch rates for those vessels that were present on both days. The line is a locally weighted regression line through the points.



However, this does not mean that the vessels that were already in the area may not be detrimentally affected by the influx of other vessels. The relationship between the number of new vessels that arrive in an area on a day and the difference in mean catch rates between the previous day and the day in question for those vessels that were present on both days is shown in Fig. 4. In the 88 cases where at least 5 new vessels arrived, the mean decreased on 43 occasions or 48.9% of the time. In the 320 cases where fewer than 5 new vessels arrived, on 161 occasions the mean (for those vessels present on both days) decreased, which is 50.3% of the time. In the 29 cases where at least 10 new vessels arrived, on 15 occasions (51.7%) the mean decreased. In the 379 cases where fewer than 10 new vessels arrived, on 189 occasions the mean decreased, which is 49.9% of the time.

So it does not seem that these vessels are detrimentally affected, but of course, we do not know what catch rates they would have got if the other vessels had not arrived. It may be that the vessels were following the movement of a group of fish, which came into the area at the same time as the new vessels. If the new vessels had not been there, the vessels that were already there might have had an even larger increase in mean catch.

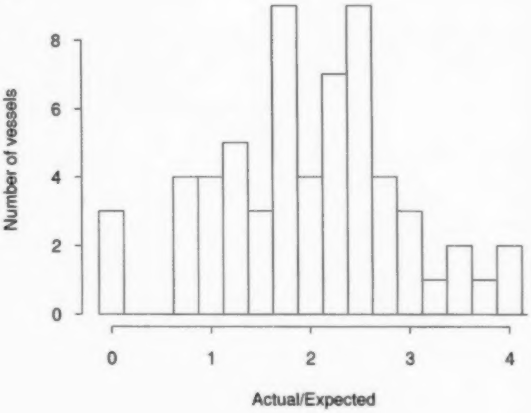
6. Different strategies for different vessels

Therefore, a potential strategy for fishing is (1) if fishing yesterday was good, fish where you fished yesterday; (2) otherwise, move to an area with lots of other vessels.

Clearly, if all vessels followed this strategy all the time, the fleet would fold up into a tiny ball, and would not have the capability to move anywhere or follow movements of the fish. So at least some of the vessels, for some of the time, must be doing something more adventurous, such as moving into an area where few or no vessels are fishing. They may choose this area on the basis of historical catch rates or by predicting the movement of the fish.

To determine whether the factors that affect how a vessel decides where to fish are the same for all vessels or whether there are groups of vessels that follow different strategies, the results given above for the "aggregation" strategy were examined on a vessel by vessel basis, to compare the actual number of times a vessel appeared to follow that strategy with the number expected if it had been behaving by chance alone. These results are shown in Fig. 5. It seems that there is a distribution of frequencies with which vessels use this strategy, but that there are not two groups of vessels, ones who use the strategy very frequently and the others who use it rarely.

Fig. 5. Histogram of 61 ratios of actual/expected occurrences of vessels moving to areas consistent with a strategy of moving to an area with at least 25% of vessels fishing there on the previous day. These ratios are taken individually for each of the 61 vessels in the fleet.



To determine whether a distribution such as this could be obtained if the vessels were in fact all following the same general strategy, but random variation caused some vessels to have greater realized ratios than others, a simulation procedure was used, as described in the Methods section.

In the 200 simulations, the 25% quantile of the simulated data set was never as low as 1.24, that of the actual distribution. The median 25% quantile in the 200 simulations was 1.59. Similarly, the 75% quantile was never as high as 2.46, the 75% quantile of the actual distribution. The median 75% quantile in the 200 simulations was 2.15.

This indicates that the distribution obtained from the assumption that all vessels are following the same strategy is much narrower than the true distribution. This suggests that vessels are not following the same strategy, and that there are some vessels that use this strategy more often than others.

Discussion

The methods described in this paper allow testing of more complicated strategies in more complicated fisheries than have been attempted previously. Eales and Wilen (1986), Hilborn and Ledbetter (1979), and Gillis et al. (1993) all tested vessel movements against a simple hypothesis in which identical vessels chose to fish in one of a small number (eight, eight, and three, respectively) of areas to maximize their profits, based on expected catch rates and costs.

This work differs from these papers in two main ways. Firstly, because the hoki fishery included a range of different fishing vessels fishing 24 h a day, it was necessary in this work to standardize the CPUE data. If this had not been done, the effects of vessel behaviour may have been obscured by other strong effects on catch rates such as time of day or type of vessel.

Secondly, by investigating the attractiveness of each area on a vessel-by-vessel as well as a day-by-day level, it was possible to test much more complicated vessel strategies against the data. For example, whether an area is nearby to an area that a vessel has been fishing in is specific to that vessel on that day. For each vessel on each day we need to evaluate not only whether the movement it made was consistent with the proposed strategy but also the probability of the vessel making a movement consistent with the strategy if it was actually behaving by chance. Any proposed strategy for which we have the same (or more) information than the fishers themselves can be tested in this way.

Eales and Wilen (1986), Hilborn and Ledbetter (1979), and Gillis et al. (1993) all found that fishers did appear to take fleet-wide average catch rates into account when deciding where to fish. In the hoki fishery however, although there is evidence that individual fishers respond in a rational way to their own catch rates, there is no evidence that there is information exchange about average catch rates on a daily timescale that is useful in deciding where to fish. Fishers either do not know what the mean catch rate of other vessels is or do not consider it important enough to make decisions based on that information, at least on a day by day basis.

This underlines the need to test hypotheses about fishing strategies explicitly in each fishery, since it is impossible to generalize results from one fishery to apply to another fishery.

Other work in this field has suggested using simulation to show what effect plausible vessel strategies would have on CPUE as a measure of abundance (e.g., Hilborn and Walters 1987). Testing these strategies against the data itself focuses the simulations on behaviours and strategies for which there is evidence that they are actually used in practice. There are strategies (such as "choose an area that had a higher than average mean catch rate yesterday") that are plausible, but for which there is no evidence in this fishery.

However, simulation or anthropological studies can yield suggestions for possible strategies that can then be tested against the data. For example, these results suggest that the concepts of risk–averse behaviours and deliberately fishing alongside other vessels observed by Andersen (1972) and Cove (1973) in Newfoundland trawler fisheries of the early 1970s have relevance to a modern industrial fishery in New Zealand in 1993. Vessels appeared to move to areas where aggregations of vessels were already fishing significantly more frequently than would be expected by chance alone.

Moreover, in the hoki fishery, it seems that not all vessels have the same mix of strategies. Some vessels use the strategy "choose an area that had at least 25% of the fleet fishing there yesterday" more frequently than others (and more frequently than would be expected by chance). The behaviour models of "stochasts" and "cartesian" introduced by Allen and McGlade (1986), therefore, seems to be a useful one. In this fishery, however, it seems that the two modes of behaviour are mostly done in different proportions by the same vessels, rather than by two completely different groups of vessels.

Of course, only a few plausible strategies have been investigated in this paper. The results raise questions about the possibility of even more complicated strategies the fishers might follow. They might, for example, take into account the catch rates of their vessel or other vessels over longer periods than 1 day. There may be greater information exchange between subgroups of the fishery, perhaps on nationality or company lines, than across the fleet as a whole. Many such hypotheses could be tested using the methods introduced in this paper.

Also, although the results are interesting, we must remember that correlation does not imply causation. It may be that vessels are moving from one area to another based on some other information that is not available for analysis, which is strongly correlated with the number of vessels present.

However, if they are interpreted correctly, the results have important implications for our use of CPUE in this fishery. CPUE data is generated not by randomly selected tows as is trawl survey data but by fishers working at their business, trying to optimize their catch rates. This work indicates that by using a strategy that deliberately exaggerates aggregation, that autocorrelation in the data set is likely to be an important problem for any linear model. In addition, the suggestion that vessel movements are poorly predicted by the true catch rate distributions, suggests that maps of vessel positions should not be taken as an indication of the density distribution of the fish.

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Appendix

To determine for each hypothesised strategy, the number of actual occurrences of vessels behaving consistently with this strategy and the number that could be expected by chance alone, the following steps are necessary.

(1) Identify all occasions in which a vessel v fished in an area A in which it did not fish the day before (identified as $d-1$).

(2) Determine whether area A was consistent with the strategy.

(3) Identify all the choices that vessel v had of possible areas it could fish in on day d to determine the probability of it going to an area consistent with the strategy simply by chance. It could either (i) fish in an area no vessel had fished in on day $d-1$, or (ii) fish in an area where there were vessels fishing on day $d-1$.

In a proportion p (approximately 15%) of movements, vessels moved to completely new areas. Since there is no information about the fishing in these areas, the vessel could not have been following a strategy such as following high mean catch rates, and there is no information with which to predict which of the many unfished areas the vessel might choose. Therefore, we simply estimate that there is a $p\%$ probability that the vessel will not follow the hypothesized strategy because it will go to a completely new area.

The other $(1-p)\%$ of the time vessels moved to areas about which there was some information. To estimate the probability that the vessel will follow the strategy by chance in this case, we need to identify the areas which would have been consistent with the strategy, and the areas which would not have been consistent with the strategy. To do this we need to (i) find all the areas in which vessels fished on day $d-1$; (ii) find all the areas that vessel v fished on day $d-1$; (iii) hence find all the areas that were fished, but that vessel v did not fish in on day $d-1$; and (iv) Identify which of these areas would have been consistent with the strategy. Let the number of areas consistent with the strategy be A_1 and the number of areas not consistent with the strategy be A_2.

(4) Hence, if vessel v was choosing an area without regard to the strategy then we would expect it to have a probability of going to an area consistent with the strategy of $A_1/(A_1 + A_2)$.

Therefore, we can estimate the expected number of times that a vessel would choose an area consistent with the strategy by chance alone.

$$E(x) = \sum_i x_i P(x_i) = \sum_i \left(p \times 0 + (1-p) \times \frac{A_1}{A_1 + A_2} \right)$$

where $\sum_i$ is a sum over all the observed vessel movements.

Given the probabilities $A_1/(A_1 + A_2)$ the standard error of the expected value can be estimated by a bootstrap procedure. In this procedure, for each vessel movement we (*i*) select a random number from a uniform distribution between 0 and 1 and (*ii*) determine whether this number is smaller than $(1-p) \times A_1/(A_1 + A_2)$ to obtain a simulated set of movements consistent or inconsistent with the strategy. The number of consistent movements can then be counted. This is a sum of a number of Bernoulli trials, but since the probability of success in each trial is not the same, the result does not exactly follow a binomial distribution.

This procedure was then repeated 2500 times, and the standard deviation of the distribution of the actual number of consistent movements from the simulations taken to be the standard error of the number of times that you would expect vessels to behave in a way consistent with the hypothesized strategy, if the vessels were actually behaving by chance.

If the number of times that the vessel actually followed the hypothesized strategy is significantly larger than the expected number by chance then we have evidence that some strategy is involved.

Chronic toxicity of waterborne thiocyanate to rainbow trout (*Oncorhynchus mykiss*)

Roman P. Lanno and D. George Dixon

Abstract: Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to thiocyanate (SCN^-) concentrations of 35, 77, 115, or 154 $mg\cdot L^{-1}$ for 16 weeks. Mortality occurred either as a chronic mortality throughout the exposure period or as mortality characterized by sudden death syndrome, which occurred immediately after biweekly weighing procedures. The chronic toxicity of SCN^- to rainbow trout was characterized by a combined diffuse hyperplastic and diffuse colloid goitre, reduced growth, and decreased hepatosomatic and splenosomatic indices and thyroxine levels compared with pair-fed control fish. Trout also developed an anemia characterized by reduced hematocrit and hemoglobin levels. Plasma SCN^- concentrations increased with ambient SCN^- levels with bioconcentration factors ranging from 9.3 to 13.4. These observations indicate that chronic SCN^- toxicity in rainbow trout is in part due to interference with normal thyroid function.

Résumé : Des truites arc-en-ciel (*Oncorhyncus mykiss*) juvéniles ont été exposées pendant 16 semaines à du thiocyanate (SCN^-) en concentrations de 35, 77, 115 ou 154 mg/L. On a observé une mortalité chronique durant la période d'exposition et une mortalité due au syndrome de mort subite, lequel survenait immédiatement après les pesées bihebdomadaires. La toxicité chronique du SCN^- chez la truite arc-en-ciel se caractérisait par une combinaison de goitre diffus hyperplasique et colloïde, une baisse de croissance ainsi que des indices hépatosomatiques et splénosomatiques et des taux de thyroxine réduits par comparaison aux poissons témoins ayant eu la même alimentation. On a aussi observé chez les sujets une anémie caractérisée par un hématocrite et un taux d'hémoglobine réduits. Les taux de SCN^- plasmatique ont augmenté avec l'élévation des concentrations dans le milieu, les facteurs de bioconcentration allant de 9,3 à 13,4. Ces observations indiquent que la toxicité chronique du SCN^- chez la truite arc-en-ciel est due en partie à une perturbation de la fonction normale de la thyroïde. [Traduit par la Rédaction]

Introduction

Thiocyanate (SCN^-) is available to aquatic organisms either directly, by uptake of waterborne SCN^-, or indirectly, by endogenous conversion of assimilated cyanide (HCN). Because SCN^- is produced during the treatment of CN^--laden mining wastes (Devuyst et al. 1982; Ganzarcyk et al. 1985; Ingles and Scott 1987), elevated SCN^- concentrations can occur in waters receiving effluent from gold ore extraction and concentration activities (Huiatt et al. 1983). An extensive literature base exists on the acute and chronic toxicity of HCN to aquatic organisms and has resulted in an Ontario water quality objective of 0.005 $mg\ HCN\cdot L^{-1}$ (Ontario Ministry of the Environment 1984). SCN^- is much less toxic than HCN; although the 96-h median lethal concentrations (LC_{50}) of HCN for fish are consistently $\leq 0.5\ mg\cdot L^{-1}$ regardless of species (Leduc 1984), the reported 96-h SCN^- LC_{50s} for salmonids range from 161 to 264 $mg\cdot L^{-1}$ (Parker 1983; Heming et al. 1985; Speyer and Raymond 1988; Heming and Blumhagen 1989).

Heming et al. (1985) found that SCN^- LC_{50} estimates for brook (*Salvelinus fontinalis*) and rainbow (*Oncorhynchus mykiss*) trout were highly variable and confounded by sudden mortalities. Fish that appeared normal would rapidly exhibit loss of equilibrium and buoyancy, gasping, flaring of operculae, darkening of the epithelium, tonic convulsions, and within minutes, cessation of opercular movement and extreme rigor. These mortalities could be triggered by strenuous exercise (chasing with a dip net for 15 s), abrupt changes in photoperiod, or increased levels of spontaneous activity. This pattern of mortality, termed sudden death syndrome (SDS), has not been reported by other researchers (Parker 1983; Speyer and Raymond 1988), although they did note significant increases in mortality of rainbow trout 15 min after the application of a manual stressor at the end of 96-h lethality tests.

While the acute toxicity of SCN^- has been reasonably well defined, little is known about its chronic toxicity. Although previous endocrinological studies provide some insight on the sublethal effects of SCN^- in fish (Singh et al. 1977; Eales and Shostak 1983), SCN^- was administered by intraperitoneal injection during these studies, thus circumventing metabolic involvement of the gills in chronic SCN^- toxicity. Physiologically, other anions such as Cl^-, Br^-, I^-, and F^- inhibit SCN^- uptake from the water by trout (Heming et al. 1985; Heming and Blumhagen 1989). Because of the extremely efficient metabolic conversion of CN^- to SCN^- (Leduc 1984), suggestions have been made that chronic CN^- toxicity may actually be due to chronic SCN^- toxicity. Sublethal effects of SCN^- in mammals have been linked to anti-

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thyroidal activity; SCN^- competitively inhibits the active uptake of I^- from the blood by the thyroid (Wolff 1964) and interferes with thyroid hormone synthesis (Yamada et al. 1974). Similar physiological effects have not been explored in fish exposed to waterborne SCN^-.

The objective of this research was to characterize the signs of chronic toxicity in juvenile rainbow trout exposed to nominal SCN^- concentrations of 40, 80, 120, and 160 $mg \cdot L^{-1}$ during a 16-week period. Because a preliminary 4-week test indicated that exposure to waterborne SCN^- resulted in a consistent reduction in feed intake, we used a pair-fed design; the study was completed in triplicate for a total of 24 tanks. Toxicity was assessed in terms of mortality, behavioural abnormalities, wet mass, feed conversion efficiency (FCE), condition factor (*k*), hepato somatic index (HSI), and spleno somatic index (SSI), hematocrit (Ht), hemoglobin (Hb), as well as plasma SCN^- and L-thyroxine (T_4) concentrations. Gross pathology and histopathology of liver, gill, kidney, spleen, and thyroid tissue were also evaluated.

Materials and methods

Juvenile rainbow trout with a mean weight of approximately 0.5 g, were obtained from Rainbow Springs Trout Farm, Thamesford, Ont. The fish were acclimated to laboratory conditions in 200-L continuous flow (1 $L \cdot min^{-1}$) tanks at 15°C for 3 weeks prior to the toxicity test. During acclimation the fish were fed a commercial trout starter formulation (No. 0.5 granule size, ≥52% protein, ≥18% lipid, ≤2.5% fibre; Martin's Feed Mills, Elmira, Ont.) ad libitum twice daily. The acclimation photoperiod, used throughout the study, was 16 h light : 8 h dark with 0.5 h of artificial dusk and dawn included in the light portion of the cycle.

The experimental exposure apparatus consisted of twenty-four 60-L aerated white fiberglass tanks, each of which received a continuous flow of water at a rate of 200 $mL \cdot min^{-1}$. Mariotte bottles (Leduc 1966) dispensed SCN^- stock solution, prepared with reagent-grade KSCN (BDH Chemicals Co., Toronto, Ont.) and deionized water, into the dilution water stream of the treated tanks to achieve the required design concentrations. The experimental system was divided into three blocks to account for daily foot traffic in the laboratory, because daily disturbances have been reported to increase mortality rates in rainbow trout exposed to SCN^- (Heming et al. 1985).

During chronic toxicity testing with fish, feed avoidance or reduced feed intake due to the presence of waterborne toxicants is a common problem, often leading to misinterpretation of growth and physiological responses (Lanno et al. 1989). One way of dealing with reduced feed intake is to use a pair-fed (equalized-feeding) design (Baker 1986; Lanno et al. 1989) where each treated tank is paired with an unexposed control tank. The fish in the control tank are fed at a rate to match the daily food consumption of the fish in the corresponding treated tank. This makes it possible to distinguish indirect effects on growth and physiology due to appetite suppression from the direct physiological effects of the toxicant. Because of a reduction in food consumption observed in a preliminary SCN^--exposure study, this experiment incorporated a pair-feeding design with each treatment concentration and its pair-fed control occurring once within a block.

A detailed characterization of the groundwater supply to our laboratory is given in Kevan and Dixon (1991). During the study temperature was measured daily, while total hardness (EDTA titration; APHA, AWWA, and WPCF 1980), alkalinity (HCl titration; APHA, AWWA, and WPCF 1980), pH (pH meter), and dissolved oxygen (Winkler titration; APHA, AWWA, and WPCF 1980) were determined weekly. The mean (SE, *n*) water conditions were temperature, 15.0°C (0.41, 110); total hardness, 382 $mg \cdot L^{-1}$ as $CaCO_3$ (12, 13); pH, 7.68 (0.03, 13); and dissolved oxygen, 6.9 $mg \cdot L^{-1}$ (0.6, 13). Water samples for the determination of SCN^- concentrations were taken on a rotating basis from the treated tanks and weekly from the control tanks. The 200-mL samples were held in linear polyethylene bottles and either analyzed immediately or frozen at −20°C pending analysis. The SCN^- concentrations were determined by measuring the optical density at 480 nm after complexation with ferric nitrate (APHA, AWWA, and WPCF 1980); the detection limit was 0.25 $mg \cdot L^{-1}$. The mean (SE, *n*) in tank assayed SCN^- concentrations were 35 (1.3, 21), 77 (2.9, 21), 115 (3.5, 18), and 154 (6.7, 15) $mg \cdot L^{-1}$; concentrations in control tank samples were consistently below the detection limit.

On the day before the start of SCN^- exposure, 55 rainbow trout were placed into each of the 24 experimental tanks. The pooled within-tank fish weights were standardized to a coefficient of variation of approximately 2.6%. At this point the fish had an overall mean weight of 1.4 g. Throughout the 16-week study the fish in each treatment tank were fed a practical trout diet (Table 1) ad libitum four times per day. The weight of the ration consumed during feeding by fish in a treatment tank was determined daily, and an equal weight of food was then fed to the matched control tank. Trout were weighed every 2 weeks to determine growth rates and to provide a routine stressor to measure the expression of SDS within each treatment population. The fish were counted into a polyethylene colander held in water. The colander was then weighed with and without fish to give (by difference) the total fish biomass. As mortalities occurred in treatment tanks, the amount of feed offered to control tanks was adjusted proportionately. Feed intake, mortalities, and feeding behaviour were monitored daily. Feed conversion efficiency (FCE) over the 16-week period was calculated as:

$$(1) \quad \mathrm{FCE} = \frac{100(\mathrm{FW}_{16} - \mathrm{FW}_0)}{11\,200\,(\mathrm{FC/FD})}$$

where FW_0 and FW_{16} are, respectively, the mean mass per fish (grams) at 0 or 16 weeks, FC is the mass of feed consumed (grams) over 16 weeks, and FD is the number of fish days (1 fish alive in the tank for 1 day = 1 FD).

At the termination of the 16-week growth study, individual fish were anaesthetized in tricaine methanesulphonate (MS 222) and killed by cervical dislocation. Mass and fork length were recorded for the calculation of condition factor (*k*) as 100 (mass/length^3) with the mass and length of the fish in grams and centimetres, respectively. Blood was collected into heparinized polyethylene centrifuge tubes by caudal peduncle severance. Subsamples were taken for the determination of Ht (by centrifugation) and Hb (Unopette cyanmethemoglobin kit; Becton-Dickinson, Rutherford, N.J.); the remainder of the blood sample was spun at 5000 × *g* for 5 min in a clinical centrifuge to isolate the plasma. The plasma was pipetted into capped polyethylene vials, frozen in liquid nitrogen (−196°C), and stored at −80°C pending analysis for concentrations of SCN^- (colorimetric determination of total cyanide-reactive substances; Lambert et al. 1975) and T4 (radioimmunoassay kit; Jolden Diagnostics, Scarborough, Ont.). Bioconcentration factors (BCF) for plasma SCN^- at 16 weeks were calculated as (plasma SCN^- concentration)/(SCN^- exposure concentration). Sample sizes for each variable are provided with the results.

The peritoneal cavity of each fish was opened and the organs were examined for abnormalities. The liver and spleen were removed and weighed to allow calculation of HSI and SSI as 100(organ mass/body mass). Portions of the liver, kidney, head kidney, gill, spleen, and the lower jaw (encompassing the thyroid tissue) were fixed in buffered formalin, decalcified (lower jaw only), dehydrated, embedded in paraffin, sectioned (5 μm), and stained with haematoxylin and eosin using standard methods. The slides were coded and randomized prior to qualitative examination by light

Table 1. Formulation of the diet fed to rainbow trout during the 16-week SCN$^-$-exposure study.

Component	%
Capelin meal (67% crude protein)	38
Soybean meal (49% crude protein)	25
Wheat middlings (18% crude protein)	22
Fish oil	12
Mineral premix	1
Vitamin premix	2

Note: The diet is similar to the GRT-70 formulation of Cho et al. (1974) and Lanno et al. (1985). The composition of the mineral and vitamin premixes are given in Cho et al. (1974).

microscopy. No samples were available from fish exposed to 154 mg SCN$^-$·L^{-1} because all fish had died by 12 weeks.

Statistical analysis

Data were analyzed in two phases using standard statistical programs (SAS Institute Inc. 1985). Initially, the data for SCN$^-$-exposed fish and pair-fed controls were examined separately using one-way ANOVA with tanks nested within treatments and subsampling from each tank. In the event of significant tank within treatment effects, type III sums of squares were used for testing treatment significance. If the ANOVA was significant, multiple means comparisons were conducted using Tukey's HSD ($\alpha = 0.05$) (Sokal and Rohlf 1981).

The data were then subjected to a two-way ANOVA, where treatment and control tanks were matched, to examine differences between SCN$^-$-exposed groups and their matched controls. Treatment and control within a block and SCN$^-$ level were the two factors in the ANOVA. The experimental design was unbalanced because of the complete mortality of one replicate of fish exposed to 115 mg SCN$^-$·L^{-1}. For this reason, type III sums of squares were used in determining the significance of ANOVAs. A significant *F*-test indicated a significant difference between SCN$^-$-exposed fish and pair-fed controls for a given response variable. If the interaction component of the two-way ANOVA was significant ($p \leq 0.05$), indicating that the difference between treatment and paired-control tanks was dependent upon SCN$^-$ level, paired t-tests ($\alpha = 0.05$) were conducted to determine differences between SCN$^-$-exposed and pair-fed control means at each SCN$^-$ level.

Data normality and heterogeneity of variance were determined by residuals analysis (Montgomery 1984). If these assumptions were violated, $\log_{10}$ or arcsine transformations were applied to satisfy assumptions prior to statistical analysis. Since variable numbers of fish were sampled from each tank within treatment, estimates of within treatment variation are given as pooled SDs of the raw data. Means within treatments are based upon $n = 3$ for 35 and 77 mg SCN$^-$·L^{-1} and $n = 2$ for 115 mg·L^{-1}.

Results

Mortality increased with increasing SCN$^-$ concentration (Table 2). At 154 mg·L^{-1}, 100% mortality occurred within 12 weeks. Fish exposed to 115 mg·L^{-1} suffered 52.7% mortality over 16 weeks, although the response was highly variable; one replicate showed 100% mortality. With that replicate excluded, the mean mortality was 29.1%. SDS was observed only during the biweekly weighing and then only at exposure concentrations ≥77 mg·L^{-1}. SDS was apparent in all replicates at concentrations ≥115 mg·L^{-1} but in only one replicate at 77 mg·L^{-1}, a replicate situated near a corner of the experimental system disturbed by laboratory traffic. With the exception of that replicate, mortalities at 35 and 77 mg·L^{-1} were minimal. Mortalities not associated with weighing failed to exhibit any signs characteristic of SDS. Complete mortality data are available in Lanno (1990). The major behavioural anomalies observed in SCN$^-$-treated fish were hyperexcitability and irritability. Fish exposed to 115 and 154 mg SCN$^-$·L^{-1}, exhibited rapid and erratic spiral swimming, usually associated with the presentation of food and attempted feeding.

Feed intake was higher in fish exposed to 35 mg SCN$^-$·L^{-1} relative to other SCN$^-$-exposed groups ($p = 0.005$) (Table 2) and a similar effect was observed among pair-fed control groups ($p = 0.011$). Feed intake was not different between matched treatment and control groups ($p = 0.059$). FCE improved in groups exposed to 115 mg SCN$^-$·L^{-1} relative to other treatment groups ($p = 0.019$), but no differences were evident among pair-fed control groups ($p = 0.820$). No difference in FCE was evident between matched treatment and control groups ($p = 0.493$), but was affected by treatment level ($p = 0.046$).

Final mass and the slopes of growth curves of fish exposed to 35 mg·L^{-1} were higher relative to other SCN$^-$-exposed groups ($p = 0.024$ and $p = 0.008$, respectively) (Table 2). A similar increase was seen in the final mass of pair-fed control groups ($p = 0.034$), but slopes of the growth curves were not different ($p = 0.247$). This difference may be attributable to the high variability of the pair-fed control groups for fish exposed to 115 mg·L^{-1}. The increase in final mass between 14 and 16 weeks in one replicate was much greater than the other. No differences in final mass or slopes of growth curves between matched treatment and control groups were evident ($p = 0.075$ and $p = 0.598$, respectively); however, the difference in final mass did change with treatment level ($p = 0.040$), but slopes did not ($p = 0.166$). Condition factor was not affected by SCN$^-$ level ($p = 0.5303$) and no differences between control and treatment means were observed ($p = 0.311$) (Table 2).

All fish exposed to SCN$^-$ exhibited varying degrees of cranial deformities (Fig. 1). Relative to controls, the head of SCN$^-$-exposed fish was smaller in relation to the body and appeared to be laterally and dorsoventrally compressed. The operculae were often shortened or crumpled, exposing the gill filaments. The severity of these signs appeared to increase with SCN$^-$ concentration. Two types of pigmentation changes were apparent in SCN$^-$-exposed fish. The first was a mottled darkening of all fish that exhibited SDS. The second, not associated with SDS, was a complete darkening of some exposed fish during feeding. The response was transitory; 15–30 min after feeding the fish returned to the light pigmentation characteristic of rainbow trout held in white tanks. These signs were never apparent in control fish.

HSI and SSI were reduced in SCN$^-$-exposed fish relative to controls (HSI, $p = 0.001$; SSI, $p = 0.001$) (Table 3, Fig. 2). For HSI, an interaction term in the two-way ANOVA ($p = 0.033$) indicated that the difference in HSI between treatment groups and their matched controls was dependent upon SCN$^-$ concentration, even though food intake was similar (Table 2). Figure 2 shows that this difference increased with SCN$^-$ exposure level. No such relationship was apparent for SSI ($p = 0.331$). Paired *t*-tests showed that all paired HSI values

Table 2. Mortalities, nutrition, and growth of rainbow trout exposed to SCN$^-$ for 16 weeks.

	SCN– concentration (mg·L^{-1})			
	35	77	115	154
Mortalities (%)				
Treatment	1.8^{a}	15.8^{a}	52.7^{ab*}	100^{b*}
	(0)	(13.4)	(41.3)	(0)
Pair-fed control	1.8	3.6	5.2	0.6
	(1.8)	(4.8)	(1.5)	(1.1)
SDS mortalities (treatment tanks only)	0^{a}	7.8^{a}	56.8^{b}	44.9^{b}
	(0)	(13.6)	(20.9)	(2.2)
Food intake (% body mass·day^{-1})				
Treatment	1.82^{a}	1.58^{b}	1.50^{b}	
	(0.092)	(0.048)	(0.004)	
Pair-fed control	1.78^{a}	1.56^{b}	1.47^{b}	
	(0.082)	(0.072)	(0.049)	
Feed conversion efficiency				
Treatment	1.16^{a}	1.17^{a}	1.34^{b}	
	(0.042)	(0.049)	(0.060)	
Pair-fed control	1.24	1.22	1.24	
	(0.059)	(0.029)	(0.016)	
Mass (g·fish^{-1})				
Treatment	15.8^{a}	11.2^{b}	11.9^{ab}	
	(1.8)	(1.2)	(0.7)	
Pair-fed control	16.9^{a}	12.2^{ab}	11.3^{b}	
	(2.6)	(1.2)	(0.4)	
Slopes of growth curves				
Treatment	0.063^{a}	0.054^{b}	0.056^{b}	
	(0.002)	(0.002)	(0.001)	
Pair-fed control	0.064	0.056	0.058	
	(0.003)	(0.004)	(0.006)	

Note: Values are means, with SDs given in parentheses, in the units of the raw data. Because of the complete mortality of one replicate prior to 16 weeks, $n = 2$ for the 115 mg SCN$^-$·L^{-1} groups; $n = 3$ for all other groups. Means in rows with a common superscript are not significantly different (Tukey's HSD, $\alpha = 0.05$). An asterisk indicates a significant difference between treatment and pair-fed control means at a given SCN$^-$ level (paired *t*-test, $\alpha = 0.05$).

were significantly different. The pair-feeding experimental design of this study provided the unique opportunity to distinguish between effects due to feed intake and those due to toxicant exposure. The reason for reduced HSI in SCN$^-$-exposed trout is not clear, but qualitative liver histology observations suggest lower lipid levels relative to control fish. Decreased HSIs may be due to an increased requirement for energy reserves such as lipid and glycogen by the fish to meet maintenance energy requirements during exposure to SCN$^-$. Although pair-feeding experimental designs can become somewhat complex, they should be applied in situations where toxicant exposure results in feed refusal or reduced feed intake and the response parameters that are monitored are directly affected by reduced feed intake.

Fish exposed to SCN$^-$ developed an anemia characterized by decreased Hb concentration ($p = 0.002$) and Ht ($p = 0.002$) relative to controls (Table 3). Neither Hb ($p = 0.567$) nor Ht ($p = 0.108$) was dependent on the level of SCN$^-$ exposure. Plasma SCN$^-$ concentrations increased with increasing SCN$^-$ exposure concentration ($p = 0.011$) (Table 3). Plasma SCN$^-$ BCFs of 13.4, 9.3, and 9.3 were evident for fish exposed to 35, 77, and 115 mg SCN$^-$·L^{-1} respectively. Plasma T_4 concentrations were lower ($p = 0.028$) in treated fish relative to controls, although no dose response was evident ($p = 0.670$) possibly because of significant variability between replicate tanks within treatments ($p = 0.042$). SCN$^-$ was detected (3.2 mg·L^{-1}) in the plasma of one control fish.

Histological changes were most prominent in the thyroid but were also evident in the liver, gill, kidney, and spleen. The thyroid tissue of control fish showed low numbers of large thyroid follicles lined with squamous to low cuboidal epithelium; most were filled with dense eosinophilic colloid (Fig. 3*a*). In contrast, all SCN$^-$-exposed fish examined showed elevated numbers of follicles (Fig. 3*b*). The follicles were small, irregular in shape and size, and some were collapsed. The follicular epithelium of treated fish was

Fig. 1. Specimens of rainbow trout representative of those exposed to 35 mg $\mathrm{SCN^{-}\cdot L^{-1}}$ (lower) or pair-fed control conditions (upper) for 16 weeks.

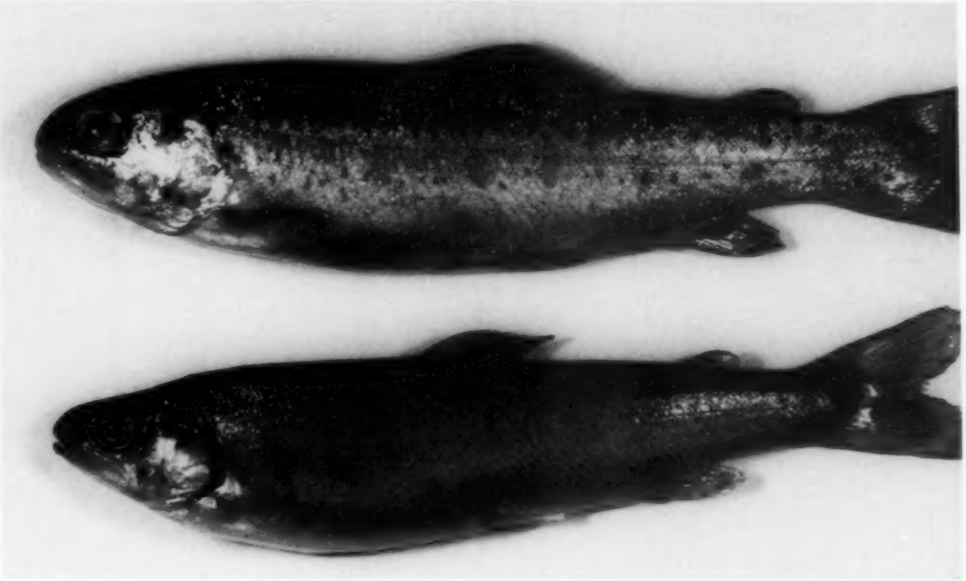
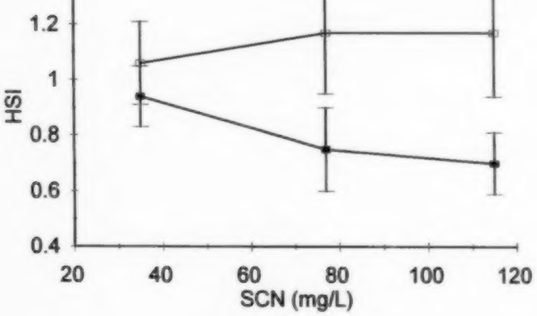


Fig. 2. Pair-feeding experimental design using HSI as an example. All treatment means (■) and matched pair-fed controls (□) are different ($\alpha = 0.05$, *t*-test). The difference between paired treatment and control means increases with $\mathrm{SCN^{-}}$ concentration, as suggested by the significant interaction term ($p = 0.0334$) in the two-way ANOVA. Error bars are pooled SDs for treatment means.



hypertrophic and hyperplastic. The lumina of approximately 30% of the follicles contained abundant eosinophilic colloid, while 30% contained scant amounts of basophilic colloid; the balance were empty. The $\mathrm{SCN^{-}}$-induced changes in the thyroid can be best characterized as a combination of diffuse hyperplastic and diffuse colloid goitre.

Gross pathological examination revealed no apparent differences between the livers of control fish and the livers of those exposed to 35 and 77 mg $\mathrm{SCN^{-}\cdot L^{-1}}$. The livers of fish exposed to 115 $\mathrm{mg\cdot L^{-1}}$, however, were pale in colour and friable. At the histological level, liver tissue from control fish showed large numbers of lipid vesicles within the hepatocytes; no vesicular lipid deposition was apparent in the livers of any $\mathrm{SCN^{-}}$-exposed fish. Moderate numbers of necrotic hepatocytes were present in treated livers but absent from controls. Low numbers of small lymphocytes were present in the gill filament epithelium of $\mathrm{SCN^{-}}$-exposed fish. Fish exposed to 115 mg $\mathrm{SCN^{-}\cdot L^{-1}}$ also exhibited a moderate increase in the number of mucous cells on lamellae, as well as an extensive increase in the number of degenerative and necrotic epithelial and chloride cells retained in the extracellular spaces separating the inner and outer layers of the lamellar epithelium. None of these gill pathologies were evident in control fish. The hemopoietic tissues (spleen and head kidney) of fish exposed to $\mathrm{SCN^{-}}$ appeared normal with abundant erythropoietic activity. The interrenal and chromaffin tissues also appeared normal relative to controls, although there was a tendency toward early asynchrony of red cell production, as well as a reduced myeloid/erythroid ratio in the head kidney of treated fish. Relative to controls, there was a moderate to mild increase in the numbers of erythroblasts in the spleen of exposed fish; the maturation of erythrocytes appeared synchronous.

Discussion

Major morphological, histological, and physiological changes were evident in rainbow trout exposed to sublethal levels of $\mathrm{SCN^{-}}$ for 16 weeks. Morphological changes included cranial cartilage deformities (Fig. 1), while the major histological

Table 3. Condition factor, splenosomatic (SSI) and hepatosomatic (HSI) indices, hematocrit (Ht), hemoglobin (Hb), plasma SCN^-, and plasma T_4 of rainbow trout exposed to SCN^- for 16 weeks and pair-fed controls.

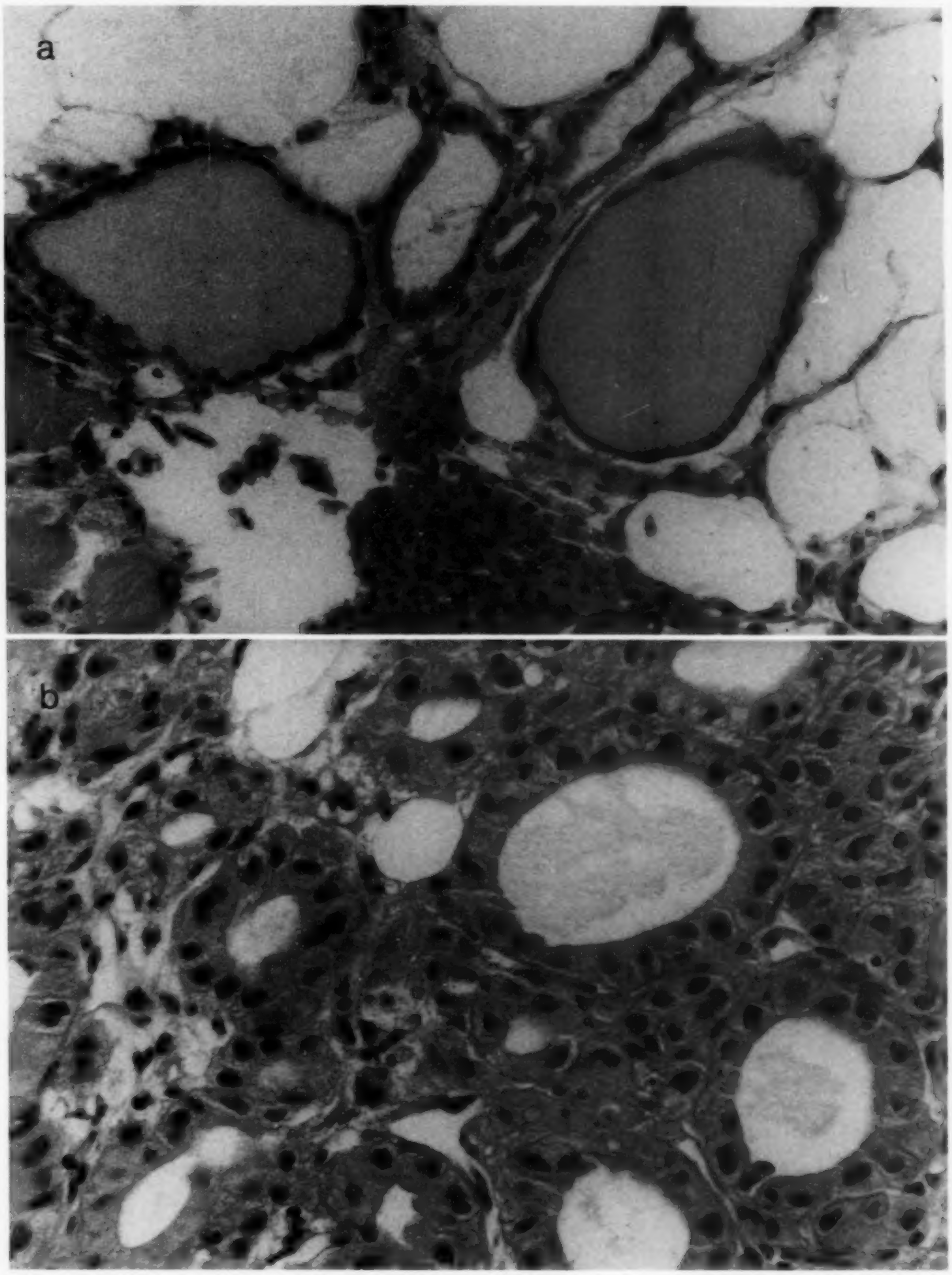
	SCN^- concentration ($mg \cdot L^{-1}$)		
	35	77	115
Condition factor			
Treatment	1.07	1.09	1.03
	(0.016, 71)	(0.180, 80)	(0.104, 46)
Pair-fed control	0.92	0.93	1.02
	(0.056, 77)	(0.092, 82)	(0.014, 45)
SSI			
Treatment	0.043^*	0.035^*	0.033^*
	(0.003, 17)	(0.007, 17)	(0.006, 12)
Pair-fed control	0.094	0.101	0.088
	(0.028, 21)	(0.023, 18)	(0.034, 12)
HSI			
Treatment	0.94^*	0.75^*	0.70^*
	(0.11, 42)	(0.15, 41)	(0.11, 24)
Pair-fed control	1.06	1.17	1.17
	(0.15, 42)	(0.22, 41)	(0.23, 27)
Ht (%)			
Treatment	24.8	24.2	17.8
	(6.9, 21)	(6.0, 21)	(7.5, 12)
Pair-fed control	38.3	36.9	37.4
	(4.3, 21)	(4.7, 24)	(4.1, 15)
Hb ($g \cdot 100\ mL^{-1}$)			
Treatment	4.49	4.62	3.73
	(1.53, 18)	(1.07, 15)	(1.44, 12)
Pair-fed control	7.91	8.34	7.80
	(0.47, 18)	(1.29, 18)	(0.56, 12)
Plasma SCN^- ($mg \cdot L^{-1}$)			
Treatment	471^a	716^b	1060^c
	(172, 9)	(272, 9)	(134, 6)
Pair-fed control	0.36	nd	nd
	(0.97, 9)	(0, 9)	(0, 9)
Treatment BCF	13.4	9.3	9.3
	(1.9, 3)	(0.97, 3)	(0.65, 2)
Plasma T_4 ($nmol \cdot L^{-1}$)			
Treatment	3.50^*	4.13^*	7.31
	(0.94, 15)	(0.60, 11)	(4.9, 10)
Pair-fed control	6.82	6.77	8.16
	(0.67, 15)	(0.75, 15)	(2.0, 10)

Note: Means are presented in the units of the raw data. Because of the complete mortality of one replicate prior to 16 weeks, $n = 2$ for the 115 mg $SCN^- \cdot L^{-1}$ groups; $n = 3$ for all other groups. Unequal numbers of fish were sampled from each tank within treatment for most parameters, so pooled SD estimates and the total number of fish sampled are provided in parentheses. Means in rows with a common superscript are not significantly different (Tukey's HSD, $\alpha = 0.05$). An asterisk indicates a significant difference between treatment and pair-fed control means at a given SCN^- level (paired *t*-test, $\alpha = 0.05$). nd, not detected (detection limit 2.0 mg $\cdot L^{-1}$).

change was a diffuse colloid goitre with hyperplasia and hypertrophy of follicular cells (Figs. 3*a* and 3*b*). Physiological changes included reduced growth (Table 2), plasma T_4 levels, HSIs, and SSIs; an anemia characterized by reduced hemoglobin and hematocrit; and increased plasma SCN^- levels (Table 3).

The threshold for 16-week mortality occurred between 77 and 115 mg $SCN^- \cdot L^{-1}$. The proportion of mortalities attributable to SDS after the weighing procedure was greatest (56.8%) in the fish exposed to 115 mg $SCN^- \cdot L^{-1}$. The lower incidence of SDS in populations exposed to 154 mg $\cdot L^{-1}$ (44.9%) may have been due to the increased acute toxicity of this level of SCN^-. Although no histological observations were made on gills of fish exposed to 154 mg $SCN^- \cdot L^{-1}$, it is possible that more direct damage to the gills may have occurred at this exposure, leading to an increased number of

Fig. 3 Section through the thyroidal region of rainbow trout: (a) control, (b) exposed to 35 mg $SCN^-\cdot L^{-1}$ for 16 weeks. 400×.



non-SDS mortalities. All SDS mortalities occurred after the weighing procedure during weeks 2–8 of the study, with no evidence of SDS after that time. Either a certain proportion of the population was susceptible to SDS, and this was manifested during the first 8 weeks of the study, or some physiological adaptation to elevated levels of SCN^- occurred within surviving fish, enabling them to control the mechanism of SDS. The expression of SDS mortalities was only observed in one replicate of fish exposed to 77 mg $SCN^-\cdot L^{-1}$, suggesting that this level of waterborne SCN^- may be near the threshold for SDS induction. Total mortality was only observed in one replicate exposed to 115 mg $SCN^-\cdot L^{-1}$ which was located near an area of heavy foot traffic and is consistent with Heming et al. (1985) who also observed increased mortality in SCN^--exposed fish that were situated near areas of increased activity; this suggests that variation in mortalities may be enhanced by proximity to external stressors.

The etiology of SDS still remains obscure, but suggestions have been made that it may be caused by the sudden conversion of endogenous SCN^- to CN^- during strenuous exercise, by disruption of osmoregulation, or by direct effects on neuromuscular function (Heming et al. 1985). Although the conversion of SCN^- to CN^- has been demonstrated in vitro using mammalian systems (Goldstein and Rieders 1953; Chung and Wood 1971), in vivo conversion of SCN^- to CN^- was insufficient to account for its toxicity in mice (Smith 1973). McMaster (1988), comparing the lethality of CN^- and SCN^- to rainbow trout using a residual oxygen demand bioassay, noted SDS and pigmentation changes in fish exposed to SCN^-, but these effects were absent in CN^--exposed fish. This suggests that CN^- is probably not responsible for the onset of SDS in trout. Eales and Shostak (1983) noted that rainbow trout injected with SCN^- (1.7–180 mg·1100 g $fish^{-1}\cdot day^{-1}$) for 8–12 days died with a characteristic flaring of operculae and dorso-ventral spinal flexure. No association of these signs was made with any manual stressors that may have been applied to the fish; therefore, comparison with SDS is difficult. The most likely etiology for SDS is the direct effect of SCN^- on neuromuscular functioning (Smith 1973; Heming et al. 1985), but the exact mechanism of induction remains unclear.

Heming et al. (1985) observed that the prevalence of SDS was closely related to plasma SCN^- levels, with expression in half the population when plasma SCN^- levels reached approximately 250 $mg\cdot L^{-1}$. Based upon this suggestion, SDS would have been expected in all SCN^- exposure concentrations in this study (Table 3). This interlaboratory variability is difficult to interpret, as only Heming et al. (1985) and Heming and Blumhagen (1989) have observed SDS and, in general, found trout to be more sensitive to waterborne SCN^- levels than other researchers, with mortalities occurring at concentrations as low as 8 mg $SCN^-\cdot L^{-1}$. Additional mortalities of fish exposed to SCN^- have been observed after the application of a manual stressor (Parker 1983; Speyer and Raymond 1988), but SDS as described by Heming et al. (1985) was only observed in the current study. Differences in susceptibility of trout to waterborne SCN^- and the development of SDS may be due to interlaboratory differences in water quality (Heming and Blumhagen 1989). Heming et al. (1985) observed that waterborne halides such as chloride (Cl^-) compete with SCN^- for uptake at the gill and suggested that elevated ambient Cl^- levels may protect fish from SCN^- toxicity, including SDS. However, of all the lethality studies with waterborne SCN^-, our laboratory water had the highest Cl^- level (108 $mg\cdot L^{-1}$) (Kevan and Dixon 1991), and SDS was still observed.

Fish exposed to 35 mg $SCN^-\cdot L^{-1}$ had significantly higher mass and growth-curve slopes relative to other SCN^--exposed groups (Table 2). While body mass of the pair-fed control groups followed a similar pattern, growth-curve slopes were not significantly different. The increase in body mass at 16 weeks coincides with increased food consumption (Table 2). Although fish exposed to 35 mg $SCN^-\cdot L^{-1}$ were larger relative to other treatments, it is not known how this growth rate would compare with control fish that were fed ad libitum. The experimental design could have been enhanced by including an ad libitum fed control to allow the full expression of phenotypic and genotypic characteristics of this strain of rainbow trout under optimum growth conditions. The lack of distinction between the slopes of the growth curves for control groups is due to the variation in growth between 14 and 16 weeks in one replicate of control fish and to the loss of one replicate of fish exposed to 115 mg $SCN^-\cdot L^{-1}$, reducing the sample size to two. Fish exposed to 115 mg $SCN^-\cdot L^{-1}$ had significantly higher FCEs relative to groups exposed to lower levels of SCN^-. Since mortalities also increased in groups exposed to 115 mg $SCN^-\cdot L^{-1}$, surviving fish may have adapted physiologically to SCN^- exposure. The increased FCE at 115 mg $SCN^-\cdot L^{-1}$ may be an expression of hormesis. There was no difference in FCE among the pair-fed control groups.

Bioconcentration factors (BCFs) for SCN^- in the plasma of trout ranged from 9.3 to 13.4, assuming that equilibrium had been reached. This is likely, since Brown et al. (1995) established that SCN^- in the plasma of rainbow trout exposed to a waterborne SCN^- level of 40 $mg\cdot L^{-1}$ reached equilibrium in 16–20 days. Brown et al. (1995) measured a much lower BCF of 1.6, possibly because of differences in methodologies for the analysis of plasma SCN^-. Plasma SCN^- in the current study was determined colorimetrically (Lambert et al. 1975), which is subject to many positive interferences, while the determination of SCN^- by Brown et al. (1995) was conducted by a modified HPLC method, which is not subject to these interferences. Heming et al. (1985) also measured plasma SCN^- using colourimetric methods, so it is likely that their estimates were also elevated.

The sublethal effects of SCN^- in mammals result from its antithyroidal activity; SCN^- competitively inhibits the active uptake of iodide from the blood by the thyroid (Wolff 1964) and interferes with thyroid hormone synthesis (Yamada et al. 1974). Sublethal SCN^- toxicity in rainbow trout also appears to involve thyroid function, as suggested by histological alterations in normal thyroid structure and reductions in plasma T_4 levels of fish exposed to SCN^-. A diffuse hypertrophic, hyperplastic goitre similar to that found in this study has also been observed in fish immersed in concentrated solutions of thiourea (TU), a potent antithyroid, for 1–12.8 weeks (Grosso 1961; Pandey and Leatherland 1970; Mukherjee 1975). The relationship of antithyroids to plasma thyroid hormones in fish has not been well defined. Waterborne exposure of rainbow trout to a 15 $mg\cdot L^{-1}$ solution of thiourea for 67 days (Eales 1981) or dietary exposure to propylthiouracil (1.5 or 6.0 $mg\cdot g^{-1}$) for 139 days (Sullivan et al. 1987) did not alter plasma T_4 or triiodothyronine (T_3) levels. Intraperitoneal (ip) injections of SCN^- (0.44 mg·100 g $fish^{-1}\cdot day^{-1}$) for 28 d (Eales and Shostak 1983) increased plasma T_4 and T_3 levels relative to controls, while ip injections of pyrazole (278 mg·$kg^{-1}\cdot day^{-1}$), a potent antithyroid in rats, initially depressed plasma T_3 but not T_4 levels in rainbow trout (Eales and Omeljaniuk 1981). Plasma T_3 levels returned to control values by 20 days, possibly indicating adaptation to that level of antithyroid. Environmental contaminants such as pentachlorophenol have been found to decrease plasma thyroid hormone levels and increase thyroid epithelial cell height (Hickie et al. 1989), as have canola-meal based trout diets (Leatherland and Hilton 1988). In the current study, plasma T_4 levels were significantly depressed in trout exposed to 35 or 77 mg $SCN^{-}\cdot L^{-1}$ relative to pair-fed controls, but no difference was found between fish exposed to 115 mg $SCN^{-}\cdot L^{-1}$ and pair-fed controls (Table 3), likely because of an increased within-treatment variance component.

The deformities in cranial cartilage development, as well as the transitory epithelial darkening, may also be indicative of thyroid dysfunction. LaRoche et al. (1966) and Norris (1969) noted a marked retardation of skull growth, flaring and reduction in the size of operculae, and darkened colouration of radiothyroidectomized rainbow trout relative to controls. However, Norris (1969) did not observe alterations in head/body proportions of radiothyroidectomized chinook salmon (*Oncorhynchus tshawytscha*), indicating the possibility of different responses between species. Comparatively, decreased thyroid function has been found to retard growth and delay skeletal maturation in rats (Krane and Goldring 1986).

The marked anemia produced by sublethal SCN^- exposure is in agreement with anemia recognized in hypothyroid humans. Generally, during thyroid hypofunction in humans, there is a normochromic, normocytic, hypoplastic anemia resulting from decreased production of circulating red cells as an adaptive response to decreased tissue oxygen needs due to the depressed metabolic state (Herbert 1986). Radiothyroidectomized rainbow trout develop an anemia characterized by reduced numbers of circulating red cells, Hb, and Ht (LaRoche et al. 1966). Superimposed upon this primary anemia due to hypothyroidism may be a secondary anemia due to a deficiency in one or more of the nutrients iron, vitamin B_{12}, or folate (Herbert 1986). During prolonged sublethal CN^- exposure, CN^- may react with the cobalt moiety of vitamin B_{12} precursors, reducing the amount of vitamin B_{12} available for physiological processes (Smith 1973). This may result in the pernicious anemia and nervous system dysfunction typical of vitamin B_{12} deficiency. Thiocyanate may also bind vitamin B_{12} precursors, either directly (Wood 1975) or as CN^- from the endogenous conversion of SCN^- (Goldstein and Rieders 1953; Chung and Wood 1971), thus assisting in the development of anemia in trout exposed to SCN^-. The depressed SSI associated with SCN^--exposed fish has not been reported previously in association with hypothyroid conditions or hypoplastic anemia in fish, therefore so its etiology remains obscure.

The chronic exposure of rainbow trout to SCN^- depresses plasma T_4 levels and results in the development of a diffuse colloid goitre, with marked thyroid epithelial cell hypertrophy and hyperplasia. The changes in thyroid morphology are consistent with increased thyroidal stimulation by pituitary production of thyroid stimulating hormone in response to negative feedback from depressed plasma T_4 levels. It is not clear whether decreased plasma T_4 levels are due to decreased synthesis or increased clearance from circulation. Similarities between the anemia and cranial deformities of rainbow trout in this study and those noted in radiothyroidectomized rainbow trout by LaRoche et al. (1966) suggest that chronic SCN^- exposure may result not only in goitre formation but the development of a hypothyroid condition. This indicates that SCN^- levels of ≥ 35 $mg\cdot L^{-1}$ are ultimately beyond the limits of physiological adaptation of rainbow trout reared under the conditions described in this study.

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Metabolic specialization of muscle during development in cold-water and warmwater fish species exposed to different thermal conditions

Cosmas Nathanailides

Abstract: Temperature and developmentally induced changes in indicators of muscle growth and metabolic capacity were investigated in a cold-water and a warmwater fish species. Tilapia (*Oreochromis mossambicus*) larvae that were reared at 20, 25, and 30°C up to 4 weeks after first feeding, grew little until the 2nd week after first feeding. Subsequently, the fish reared at 25 and 30°C exhibited increased growth rates. Atlantic salmon (*Salmo salar*) that were reared at ambient temperature (4.3°C up to hatching, 9.7°C from hatching to first feeding, and 10.6°C to 3 weeks after first feeding) grew faster than salmon reared at 11°C. In both species, carcass cytochrome *c* oxidase (CCO) activity increased rapidly with development or growth. In salmon, lactate dehydrogenase (LDH) activity increased with development whereas in tilapia it decreased. In the white muscle of tilapia and the red muscle of salmon, the density of mitochondria increased with development. Myofibre size increased with growth in tilapia irrespective of temperature but the number of myofibres increased only at 25 and 30°C. At the low temperature for tilapia fry (20°C) and at ambient temperature for salmon larvae, CCO activity and red muscle mitochondrial density was not higher than in fish reared at increased temperature.

Résumé : On a étudié chez des poissons d'eau froide et d'eau chaude les changements d'indicateurs de la croissance musculaire et de la capacité métabolique causés par des facteurs de température et de développement. Des larves de tilapia (*Oreochromis mossambicus*) ont été élevées à 20, 25 et 30°C jusqu'à 4 semaines après le début de l'alimentation : leur croissance a été faible jusqu'à la seconde semaine après le début de l'alimentation. Par la suite, la croissance des poissons élevés à 25 et 30°C s'est accélérée. Les saumons de l'Atlantique (*Salmo salar*) élevés à la température ambiante (4,3°C jusqu'à l'éclosion, 9,7°C de l'éclosion jusqu'au début de l'alimentation et 10,6°C pendant les 3 semaines suivantes) ont eu une croissance plus rapide que les saumons élevés à 11°C. Chez les deux espèces étudiées, l'activité cytochrome *C*-oxydase (CCO) mesurée dans la carcasse a rapidement augmenté avec le développement ou la croissance. Chez le saumon, l'activité lactate-déshydrogénase (LDH) a augmenté avec le développement, tandis que chez le tilapia, elle a diminué. Dans le muscle blanc du tilapia et le muscle rouge du saumon, la densité des mitochondries a augmenté avec le développement. La grosseur des myofibrilles s'accrue avec la croissance chez le tilapia et ce, quelle que soit la température, mais leur nombre n'a augmenté qu'à 25 et 30°C. À basse température, dans le cas des alevins de tilapia (20°C), et à la température ambiante, dans celui des larves de saumon, l'activité CCO et le densité des mitochondries dans les muscles rouges n'étaient pas plus élevés que chez les poissons élevés à température plus haute.
[Traduit par la Rédaction]

Introduction

The phenomenon of cold acclimation has been documented in juvenile fishes that are considered to be eurythermal and in some that are considered to be cold stenothermal (Guderley 1990; Guderley and Gawlicka 1992). After cold acclimation, several species exhibit adaptive responses including increased red muscle volume (Johnston and Lucking 1978), increased myofibrillar ATPase activity (Heap et al. 1985), increased density of mitochondria (Eggington and Sidell 1989), and increased activity of aerobic enzymes (Hazel and Prosser 1974; Guderley 1990).

In early life stages of fish, temperature can affect the rate of development, meristic features (Klinkhart et al. 1987) myogenesis, and muscle ultrastructure (Stickland et al. 1988; Vieira and Johnston 1992). In developing embryos, a metabolic reorganization can be seen (Herman and Tootle 1964). For example, in developing fish, some changes in the aerobic and anaerobic metabolic capacity can occur (Boulekbache 1981; Forstner et al. 1983; Hinterleitner et al. 1987, 1989).

Atlantic salmon (*Salmo salar*) is a cold-water fish species that exhibits reduced larval growth and muscle fibre hyperplasia when embryos are incubated at 11°C compared with fish incubated at the stream's ambient temperature

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(Nathanailides et al. 1995*a*). If it is assumed that an optimum temperature of larval growth exist for each species and that muscle fibre hyperplasia is evident in fast-growing fish (Weatherley et al. 1979), then the influence of rearing temperature on muscle fibre hyperplasia may vary with species. Indeed, elevating the incubation temperature of Atlantic herring (*Clupea harengus*) above 10°C resulted in increased muscle fibre hyperplasia (Vieira and Johnston 1992), which contrasts with the results obtained with salmon (Stickland et al. 1988; Nathanailides et al. 1995*a*).

Atlantic salmon and tilapia (*Oreochromis mossambicus*) have different reproductive strategies and thermal optima. The former has different thermal optima for each stage of development (Peterson et al. 1977) but thrives at temperatures below 20°C, while tilapia (a tropical species) can survive at temperatures above 35°C (Allanson and Noble 1964) and exhibits optimal larval growth between 25 and 30°C (Price et al. 1985). Atlantic salmon bury their eggs in the gravel of cold rivers whereas tilapia are mouth breeders that spawn at warm temperatures. Nevertheless, after hatching, the developing fish of both species exhibit a phase characterized by limited movements followed by a progressive increase in swimming activity. After hatching, salmon larvae spend a prolonged period buried in the gravel and become active towards the end of endogenous feeding. Similarly, after hatching, tilapia larvae are kept in the mouth cavity of the female and only progressively become independent. It would be reasonable to hypothesize that there is metabolic reorganization in the muscle of developing salmon and tilapia throughout these stages of development. Temperature can affect the developmental rate of muscle fibres with different metabolic properties in carp (*Cyprinus carpio*) (Nathanailides et al. 1995*b*).

The aim of this study was to compare the influence of thermal conditions on indicators of axial muscle metabolic properties such as mitochondrial density, activity of enzymes, and their relationship with somatic growth and muscle growth (increase in the size or number of myofibres) during development in a warmwater (tilapia) and a cold-water (Atlantic salmon) fish species.

Materials and methods

Tilapia

Larvae and fry were used for structural and biochemical analysis of lateral muscle in developing tilapia. For juveniles, only assays of enzyme activity were carried out (see below). Newly hatched tilapia larvae (obtained from a single pairing) were divided into three groups and kept at 25°C in 120-L plastic tanks. Upon first feeding, which occurred about 5 days after hatching, the temperature was adjusted at 0.5°C per hour to attain within 2 days three temperatures: 20, 25, and 30°C. The water in each tank was aerated; oxygen saturation was about 85–90% throughout the experiment.

Larvae were fed initially with hatching nauplii of *Artemia salina* and within 2 days after first feeding with commercial tropical larvae and fry food. Six samples were taken from each group, weekly up to the 4th week after first feeding. The fish were blotted dry with filter paper and the body mass was recorded using a precision electronic balance (Sartorius, Model 1712).

Five juveniles of tilapia (length 6.1 ± 0.15 cm; mean ± SE were acclimated at 20 or 30°C for 5 weeks and fed with commercial food for tropical fish.

Atlantic salmon

The experimental conditions were described in Nathanailides et al. (1995*a*). Briefly, all eggs were obtained from one pair of Atlantic salmon (a 2+ female and a 1+ male) that originated from the river Almond, Perthshire, Scotland. The fertilized eggs, larvae, and fry were maintained at the Almondbank salmon hatchery station, SOAFD, Freshwater Fisheries Laboratory, Pitlochry, Scotland. After fertilization, the eggs were divided into two groups; each group was placed on Perspex salmon egg incubating trays kept in plastic tanks. One group (ambient) was kept at the river temperature of the Almondbank hatchery (where the salmon of the local stock spawn). The other group was gradually heated to 11°C over 6 days and maintained at that temperature throughout the experiment. Ambient temperature was less than 4.3°C up to hatching, 9.7 from hatching to first feeding, and 10.6 to 3 weeks after first feeding (see Fig. 1 of Nathanailides et al. (1995*a*)).

Homogenates

Three to five salmon were sampled for the assay of muscle enzymes on hatching and 3 weeks after first feeding. Larvae and fry of tilapia were sampled each week (three fish from each temperature group). Fish were killed by decapitation and brain destruction, and the guts and all the fins were removed. Carcass homogenates (5% w/v) were prepared in an ice-cold medium containing 150 mM KCl and 5 mM MOPS buffer (pH 7.4), using a glass homogenizer tube fitted with a motor-driven teflon pestle and kept ice cold. Crude homogenates were used for enzyme assays because the use of centrifugation (to clarify the homogenate) can result in some loss of enzyme activity by binding to subcellular mitochondrial particles (Vezina and Guderley 1991; Tyler and Nathanailides 1995). Prior to enzyme assays, homogenates were treated with 0.05% (v/v) Triton X-100 (final concentration).

The five juvenile tilapia were killed by decapitation, the whole skeletal muscle of one side was removed, and 5% (w/v) homogenates were prepared as described above.

Enzyme assays

All assays were performed in duplicate at 20°C using freshly prepared homogenates obtained on the day of sampling. Optimum conditions for each enzyme were established during initial experiments. Cytochrome *c* oxidase (CCO) activity was assayed according to Tyler and Nathanailides (1995) in a medium containing 0.075 M potassium phosphate buffer (pH 6.8) and 0.0025 mM ferrocytochrome *c*. Ferrocytochrome *c* was prepared according to Sidell (1980). Lactate dehydrogenase (LDH) activity was assayed in a medium containing 50 mM potassium phosphate buffer (pH 7.0), 0.15 mM NADH, and 0.60 mM sodium pyruvate. All reagents were obtained from Sigma Chemical Co., St. Louis, Mo.

Ultrastructure

The whole bodies posterior to the vent of three to five larvae or fry of each temperature group were fixed in Karnovsky's fixative (4% paraformaldehyde and 2.5% glutaraldehyde in 0.15 M cacodylate buffer, pH 7.4) at 4°C as described by Sanger et al. (1990), postfixed in 1% osmic acid, dehydrated in a graded ethanol series, and embedded in resin (TAAB, Berkshire, England). Ultrathin transverse sections, obtained with a Reichert ultramicrotome, were stained with uranyl acetate and lead citrate, and electron micrographs were taken with a JEOL 100-SX electron microscope. The density of mitochondria was estimated from five red and five white muscle fibres per tissue block, obtained from three to five fish from each temperature group at each stage, using the following equation: $V_v = V_o/V_t = (P_o/P_t) \times 100$ (Weibel 1979), where V_o is the volume of mitochondria, V_t is the total volume of a muscle fibre (excluding the nuclei), P_o is the number of test points falling on the mitochondria, and P_t is the number of test points falling on the muscle fibre.

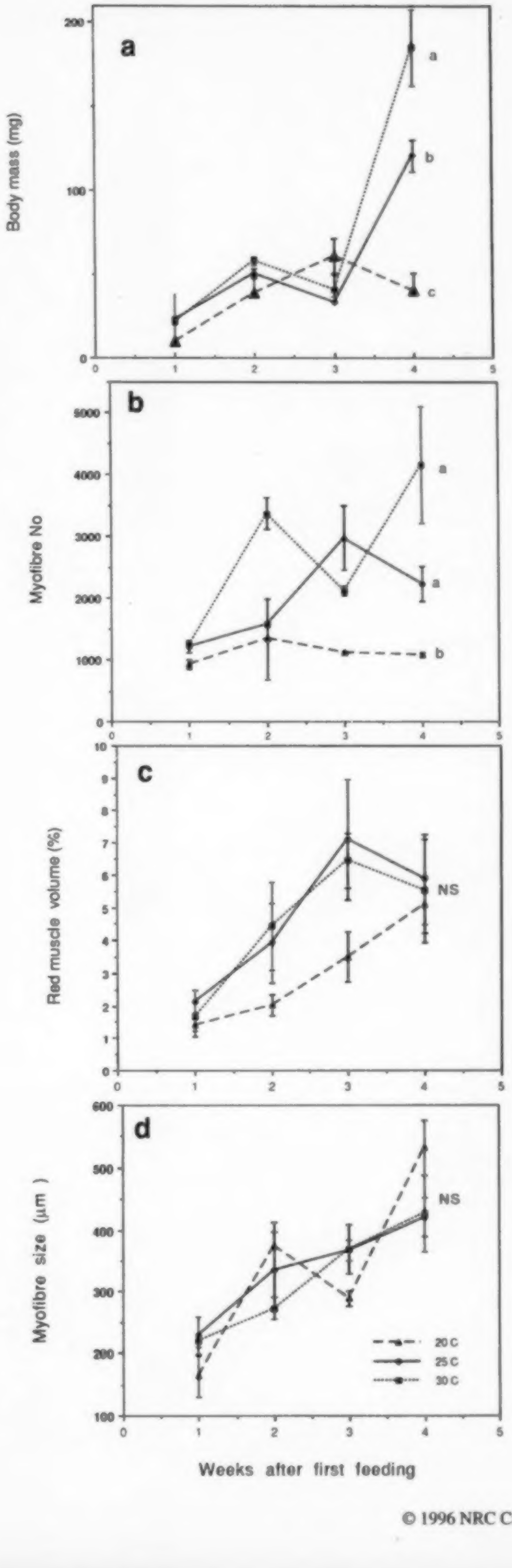


Fig. 1. Anatomical characteristics of developing tilapia reared at 20°C (broken lines), 25°C (solid lines), and 30°C (dotted lines): (*a*) body weight; (*b*) number of myofibres in a cross section; (*c*) red/white muscle cross-sectional area (%); and (*d*) mean myofibre size. Error bars show the SE. Values with different letters are significantly different ($p < 0.05$.) NS, not significant.

Muscle morphometry of tilapia white muscle

Transverse sections of 0.5–1.0 µm thickness were obtained from three larvae or fry of each temperature group, using a Reichert ultramicrotome. The sections were treated with a saturated solution of ethanolic KOH for partial removal of resin and stained according to Ontell (1974). The cross-sectional area of white muscle fibres ($n >$ 100) from the upper epaxial region in each sample was measured by light microscopy. All measurements were made using a computerized image analysis system (Seescan Ltd, U.K) linked to an Olympus (Olympus BH-2) light microscope. The total cross-sectional area of red and white muscle was traced on the screen for one side of the transverse section. Care was taken to exclude fin muscle fibres and the skin. The theoretical total number of myofibres per cross section was estimated according to Higgins and Thorpe (1990) from the ratio of total white muscle area/mean myofibre size. The volume of red and white muscle per cross-sectional area was traced on one side of the fish from three sections per group weekly. All sections that were used for muscle morphometry were obtained at the level of the vent.

Statistics

Significance of difference of the variables under investigation at each stage was examined between the temperature groups using a Student's *t*-test or one-way or two-way ANOVAs (MINITAB, version 7.2).

Results

Growth and muscle characteristics

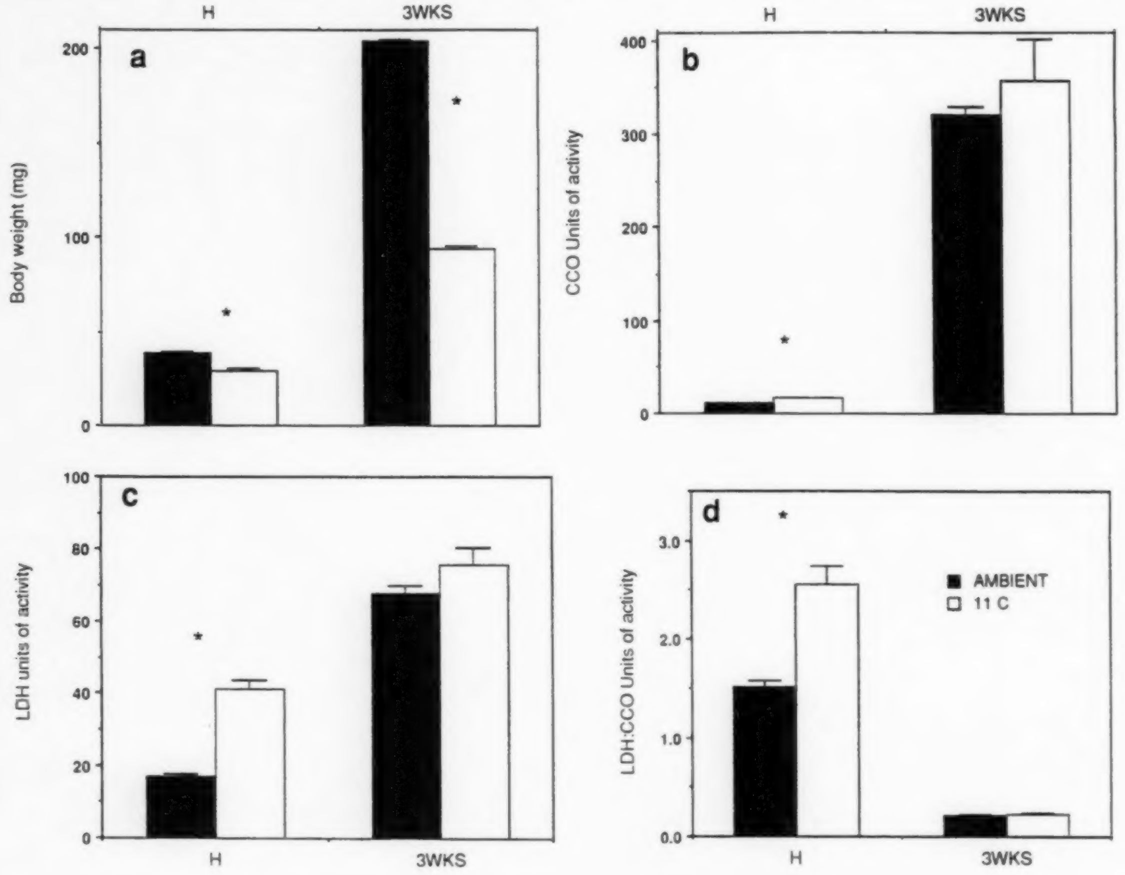
For tilapia larvae reared at 25 and 30°C, growth was initially slow but increased noticeably at about the 3rd week after first feeding. By the 4th week, the 25 and 30°C group had higher ($P < 0.05$) growth rates than the 20°C group, which showed little growth throughout the study (Fig. 1*a*). For Atlantic salmon, both at hatching and 3 weeks after first feeding, salmon of the ambient temperature group were larger than the fish reared at 11°C. (Fig. 2*a*).

Myofibre size was not significantly different between the 20 and 30°C groups, on the 1st and the 4th week after first feeding of tilapia (Table 1). The mean myofibre size tended to increase with development irrespective of thermal conditions. However, the number of muscle fibres per fish was significantly higher in the 30°C group than the 20°C group (Fig. 1*b*). The ratio of red to white muscle increased with development in all three temperature groups (Fig. 1*c*).

In tilapia, red muscle mitochondrial density (*V*mit; %) was not affected by temperature or by development. By contrast, the volume density of white muscle mitochondria increased with development. This was most marked at 30°C so that by the 4th week after first feeding the fish of the 30°C group exhibited a significantly higher mitochondrial density in white muscle than those at 20°C (Table 1).

The mitochondrial content of white muscle was significantly higher in the ambient group on hatching, but 3 weeks after first feeding it was similar in both groups (Table 2). Red muscle mitochondrial density was similar in both groups on

Fig. 2. Influence of rearing thermal conditions on Atlantic salmon: (*a*) somatic growth; (*b*) CCO activity (μmol cytochrome *c* oxidized·min^{-1}·g^{-1} muscle); (*c*) LDH activity (μmol NADH·min^{-1}·g^{-1} muscle); and (*d*) the ratio of LDH:CCO activity in the lateral muscle of developing salmon reared at ambient temperature (solid bars) and at 11°C (open bars). H, hatching, 3WKS, 3 weeks after first feeding. Asterisks indicate a significant difference ($p < 0.05$) ($n = 3$ fish per group on hatching and $n = 5$ fish per group 3 weeks after first feeding).



hatching, but it increased with development at 11°C, so that there were more mitochondria in the red muscle fibres of the 11°C group (Table 2).

Enzymes

For tilapia larvae the activity of CCO and LDH increased with development (ANOVA, $p < 0.05$) in all three temperature groups (Figs. 3*a*–3*b*). At no time was there a significant difference in the CCO activity among the three temperature groups (ANOVA, $p > 0.05$). The CCO activity (micromoles of cytochrome *c* oxidized per minute per gram muscle) of tilapia juveniles ($n = 5$ per temperature group) was not affected by temperature acclimation (48.89 ± 2.09 at 20°C, and 46.11 ± 2.94 at 30°C) whereas LDH activity (micromoles of NADH per minute per gram muscle) was significantly higher ($p < 0.05$) in the 20°C group (181.71 ± 3.82 at 20°C and 266.57 ± 32.59 at 30°C). The ratio of the LDH:CCO activity did not significantly differ between tilapia juveniles acclimated at 20°C and 30°C (3.82 ± 0.10 at 20°C and 5.20 ± 0.72 at 30°C).

For Atlantic salmon, the carcass activities of CCO and LDH and the ratio of lateral muscle LDH:CCO activity on hatching, were higher in the 11°C group (Figs. 2*b*–2*d*). In both temperature groups, CCO and LDH increased with development but LDH activity increased more in the ambient group (Figs. 2*b* and 2*c*). As a result, 3 weeks after first feeding, the LDH:CCO ratio was not significantly different between the temperature groups (Fig. 2*d*).

CCO activity correlated with body mass in salmon reared at ambient temperature ($r = 0.970$, df = 6, $p < 0.001$) and at 11°C ($r = 0.742$, df = 6, $p = 0.035$). The activity of CCO of tilapia reared at 20°C did not correlate with body mass ($r = 0.431$, df = 10, $p = 0.161$), whereas for tilapia reared at 30°C, CCO activity correlated with body mass ($r = 0.793$, df = 10, $p = 0.002$). LDH increased in both groups of salmon (Fig. 4*c*). There was a good correlation between LDH activity and body mass in developing salmon reared at ambient temperature ($r = 0.965$, df = 6, $p < 0.001$) and at 11°C ($r = 0.717$, df = 6, $p = 0.045$). In developing tilapia, LDH activity increased up to the 4th week (Fig 4*d*). LDH activity correlated with body

Table 1. Mitochondrial volume densities (V_{mit}; %) and mean myofibre size of axial muscle fibres of developing tilapia at 20 and 30°C (n = 3 fish in each group).

	20°C	30°C
Red muscle V_{mit} (%)		
Week 1	33.71 (5.04)^{a}	32.70 (4.25)^{a}
Week 4	39.73 (5.53)^{a}	35.72 (5.53)^{a}
White muscle V_{mit} (%)		
Week 1	2.93 (0.33)^{b}	2.63 (0.10)^{b}
Week 4	4.55 (0.40)^{c}	6.72 (0.87)^{d}
Myofibre size (μm^2)		
Week 1	182.20 (55.91)^{e}	219.57 (16.72)^{e}
Week 4	532.83 (69.53)^{f}	427.10 (96.3)^{f}

Note: Values are means with SEs given in parentheses. Values with the same letter are not significantly different (ANOVA).

Table 2. Mitochondrial volume densities (V_{mit}; %) of lateral muscle fibres of developing salmon at hatch and 3 weeks after first feeding incubated at ambient temperatures or at 11°C (n = 5 fish in each group).

	Ambient	11°C
Red muscle V_{mit} (%)		
Hatch	28.52 (0.30)^{a}	27.58 (1.61)^{a}
3 weeks	37.52 90.38)^{b}	46.51 (1.67)^{c}
White muscle V_{mit} (%)		
Hatch	5.50 (0.38)^{d}	3.99 (0.27)^{e}
3 weeks	4.77 (0.38)^{d}	4.91 (0.26)^{d}

Note: Values are means with SEs given in parentheses. Values with the same letter are not significantly different (ANOVA).

mass at 20°C (r = 0.736, df = 10, p = 0 .006) and at 30°C (r = 0.788, df = 10, p = 0.002).

Discussion

The thermal sensitivity of growth and muscle fibre hyperplasia varied between the two species. The lower growth rate of tilapia at 20°C, which is a low temperature for this tropical species (Brutton and Bolt 1975), than at 30°C (Fig. 1*a*) was associated with relatively less muscle fibre hyperplasia (Fig. 1*b*). By contrast, reduced muscle hyperplasia of embryonic salmon, which is associated with reduced somatic growth, is exhibited at a warm temperature (11°C) for this species (Nathanailides et al. 1995*a*).

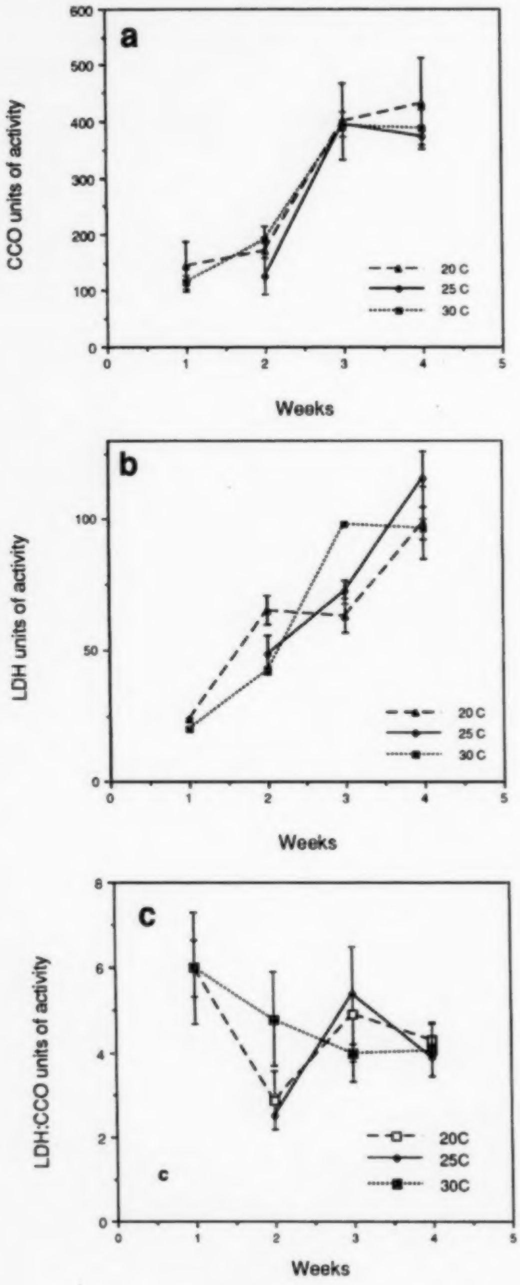
The rapid increase in the growth rate of tilapia of the 25 and 30°C group seen in the 3rd week after hatching (Fig. 1*a*), indicates that once the fry become independent and more active swimmer, growth is accelerated (personal observation). This is contrasted with Atlantic salmon, where growth rate did not increase after first feeding (Nathanailides et al. 1995*a*), i.e., when salmon fry become active swimmers (salmon embryos are not active swimmers prior to first feeding). This variation between the two species may be due to space limitations in the mouth cavity of tilapia, which possibly prompted an evolutionary adaption for an initially slower growth rate in tilapia compared with salmon.

The activity of the respiratory chain enzyme CCO is related to mass-specific oxygen consumption in fish (Smith and Chong 1982) and mammals (Simon and Robins 1971). For this reason, CCO activity has been used as an indicator of the capacity for aerobic tissue metabolism (Lind 1992) and growth (Goolish and Adelman 1987; Houlihan et al. 1993), whereas LDH activity indicates the potential for anaerobic metabolism (Lind 1992).

As in other fish species, the mass-specific oxygen consumption of tilapia reaches a peak after hatching and decreases with development in juvenile stages (De Silva et al. 1986). Indeed, activity of CCO increased with development in larval and fry stages, whereas tilapia juvenile exhibited a lower activity of CCO (Figs. 2*a* and 3*a*). This can be attributed, at least partially, to a negative correlation between aerobic metabolism and mass seen in tilapia juveniles (Caulton 1977, 1978; Job 1969) and in other fish (Pelletier et al. 1993). In support of this, in marine fish species, CCO activity decreases whereas LDH activity increases with size in adult stages of fish (Somero and Childress 1980). By contrast, in the young larval stages used in the present study, activity of LDH and CCO increased and this increase can be seen in all temperature groups. Activity of CCO in tilapia was not correlated with body mass at 20°C; however, in both species the relationship of body mass with CCO and LDH activity differs with temperature, suggesting that changes in the metabolic capacity of axial muscle in very young stages were primarily developmentally driven. In addition to this, the temperature-induced variation in the growth rate also affected the metabolic capacity of axial muscle.

After hatching in salmon (Usher et al. 1994) and on the 4th week after first feeding in tilapia (present study), the red muscle content of lateral muscle was not affected by thermal conditions. In tilapia, 4 weeks after first feeding, the fish of the 30°C group exhibited a higher density of white muscle mitochondria, yet the total lateral muscle CCO activity did not differ significantly between the two temperature groups. The higher white muscle V_{mit} of the 30°C group was not reflected in the whole muscle (red and white) activity of CCO. Moreover, in salmon, the increase in carcass CCO activity was much larger that one would expect by the increase in the V_{mit} in muscle fibres. This may be due to limitations of the stereological analysis which was restricted to cross sections obtained at a specific region; red muscle content (Greer-Walker 1970) or the ultrastructure of myofibres (Egginton and Cordiner 1995) could vary as a function of the position in the body. Furthermore, carcass muscle contains red muscle fibres associated with the fins as well as pink muscle fibres located between the red and white muscle. For this reason, carcass CCO activity should reflect changes in the metabolic properties of the entire differentiating muscle tissue in developing fish more accurately than the mitochondrial volume density. Furthermore, changes of the V_{mit} at the caudal region of the vent, which is the point of maximal flexure, may reflect changes in the usage of muscle at this anatomical region (Holloszy 1967; Baldwin et al. 1972). After hatching, salmon larvae exhibit some burst swimming, probably using the white muscle, but are not active swimmers. The swimming style of salmon after first feeding was characterized by rapid

Fig. 3. Enzyme activities in the lateral muscle of developing tilapia reared at 20°C (broken line), 25°C (solid line), and 30°C (dotted line): (*a*) CCO activity (μmol cytochrome *c* oxidized·min^{-1}·g^{-1} mass); (*b*) LDH activity (μmol NADH reduced·min^{-1}·g^{-1} mass); and (*c*) the ratio of LDH:CCO. Error bars show the SE.



burst moves associated with food capture. By contrast, from the 1st week after first feeding of tilapia, an ability for both continuous slow and rapid swimming during feeding, which is characteristic of post-larval stages (Batty 1984), was exhibited (personal observation).

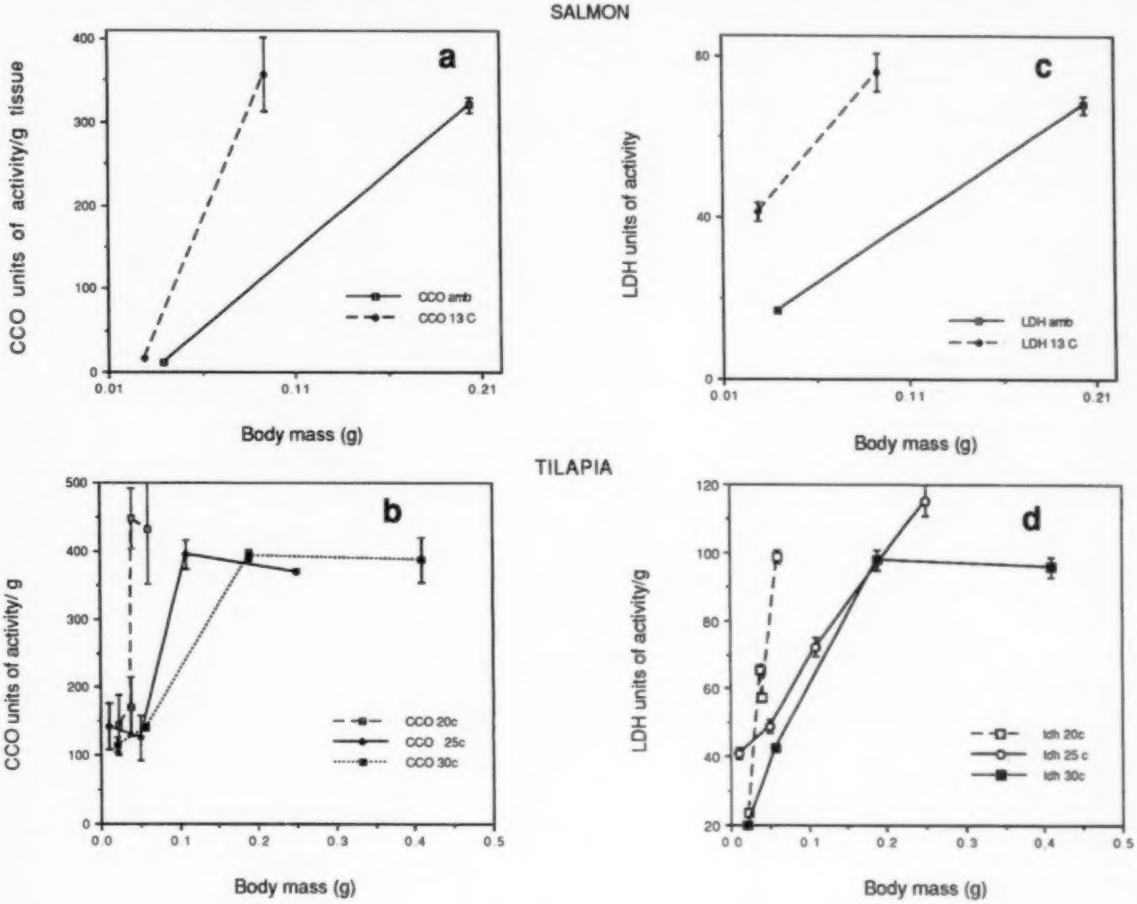
Most fish, when exposed to a rapidly cooling thermal environment, initially decrease their metabolic rate, which may recover after a period of cold acclimation, towards the original rate (Evans et al. 1962). Oxygen uptake of developing larvae in another tilapia species (*Oreochromis niloticus*) exhibits a Q_{10} between 2.2 and 3.4, with older fish exhibiting higher values (De Silva et al. 1986; Fernades and Rantin 1989). CCO activity of fish reared under different thermal conditions assayed at 20°C indicated that the enzyme content was not affected by temperature. However, because of the Q_{10} effect, which is about 1.4 for CCO from rainbow trout (*Oncorhynchus mykiss*) (Guderley and Gawlicka 1992), the actual in vivo rate of chemical reactions would vary with temperature.

The lack of a temperature-induced change in CCO activity of developing salmon and tilapia suggests that thermal acclimation is not exhibited in early larval and fry stages. Juveniles of *O. mossambicus* also did not respond to the low temperature encountered in their natural habitat in a way that nontropical species do. This implies that this tropical species may have lost or never possessed the ability to exhibit a compensatory response to cold acclimation in the way that juveniles of eurythermal fish species from temperate zones (Guderley 1990) do.

The ratio of LDH:CCO seen in salmon incubated at 11°C is higher that the ambient temperature group. Also, tilapia juveniles acclimated at 20°C have a higher ratio of LDH:CCO. Changes in the relative rates of mitochondrial enzyme activity and LDH can be related with changes in the capacity for aerobic and anaerobic metabolism (Hochacka et al. 1983; Hansen and Sidell 1983). Consequently, a higher LDH:CCO ratio may reflect an increased capacity for anaerobic metabolism under conditions of restricted aerobic ATP synthesis. LDH was also increased in cold acclimated chain pickerel (*Essox niger*) (Klecker and Sidell 1985).

Reduced growth and muscle fibre hyperplasia may be related with restricted capacity for growth through aerobic metabolism in both species. At 11°C in salmon, the rate of development was accelerated, but muscle fibre hyperplasia was not. The rate of embryonic metabolism is increased with incubation temperature, but the total oxygen consumption, from fertilization up to hatching, of Atlantic salmon (Hamor and Garside 1979) and alpine charr (*Salvelinus alpinus*) (Gruber and Wieser 1983) is temperature independent. Salmon embryos rely on inward oxygen diffusion and outward carbon dioxide diffusion through the zona radiata for the exchange of respiratory gasses. Atlantic salmon embryos exhibit a pectoral fin flutter, which helps to increase exchange of respiratory gasses through the developing gills (Peterson et al. 1991). Oxygen consumption of salmon embryos Q_{10} is 3.66 (Hamor and Garside 1979) and the Q_{10} of the pectoral fin flutter is 2.02 (Peterson et al. 1991). In addition, salmon embryos exhibit movements which increase with temperature (Klinkhart et al. 1987). In agreement with this, Hamor and Garside (1979) suggested that, as temperature increases, less of the oxygen molecules diffused in the ova are available for tissue growth. This indicates that there is a restricted capacity

Fig. 4. Changes in CCO activity (μmol cytochrome *c* oxidized·min^{-1}·g^{-1} muscle) and body weight of developing salmon (*a*) and tilapia (*b*) and LDH activity (μmol NADH·min^{-1}·g^{-1} muscle) of developing salmon (*c*) and tilapia (*d*). See Results for correlation values.



for growth through aerobic metabolism in the 11°C group compared with the ambient group, and this difference results in reduced growth. In fact, hypoxic conditions can result in smaller size of hatched salmon (Hamor and Garside 1977).

Restricted capacity for growth through aerobic metabolism can result in energy restriction. Cell division is reduced when energy supply is reduced, whereas cell growth may not (Cheeck and Hill 1970).

Equally restricted can be the aerobic capacity of post-hatched tilapia reared at 20°C because of the Q_{10} effect of temperature on the rate of chemical reactions, which would require increase in the activity of several enzymes including CCO to maintain the aerobic capacity achieved at higher temperatures. Hence, it can be implied for both species that muscle fibre hyperplasia is significantly higher in the fish that do not appear to have restricted capacity for aerobic metabolism.

The results indicated that, although some larval stages of development can proceed irrespective of thermal conditions, at the coldest temperature that larvae of *O. mossambicus* encounter, myofibre hyperplasia was impaired, whereas in a warmer thermal regime, muscle hyperplasia increased rapidly with development. By contrast in Atlantic salmon, increased temperature resulted in reduced larval muscle fibre hyperplasia (Nathanailides et al. 1995*a*). In turn, fish size, metabolic organization, and size and number of myofibres can affect the subsequent capacity for survival and growth and influence the recruitment into wild stocks.

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Contents (concluded) / Table des matières (suite et fin)

Mark A. Brobbel, Michael P. Wilkie, Kevin Davidson, James D. Kieffer, Alex T. Bielak, and Bruce L. Tufts	**2036–2043**	Physiological effects of catch and release angling in Atlantic salmon (*Salmo salar*) at different stages of freshwater migration
Joann F. Cavaletto, Thomas F. Nalepa, Ronald Dermott, Wayne S. Gardner, Michael A. Quigley, and Gregory A. Lang	**2044–2051**	Seasonal variation of lipid composition, weight, and length in juvenile *Diporeia* spp. (Amphipoda) from lakes Michigan and Ontario
Rick E. Marks, Francis Juanes, Jonathan A. Hare, and David O. Conover	**2052–2057**	Occurrence and effect of the parasitic isopod, *Lironeca ovalis* (Isopoda: Cymothoidae), on young-of-the-year bluefish, *Pomatomus saltatrix* (Pisces: Pomatomidae)
Richard R. Doucett, Geoffrey Power, David R. Barton, Robert J. Drimmie, and Richard A. Cunjak	**2058–2066**	Stable isotope analysis of nutrient pathways leading to Atlantic salmon
Feng Lin and Konrad Dabrowski	**2067–2075**	Effects of sperm irradiation and heat shock on induction of gynogenesis in muskellunge (*Esox masquinongy*)
S.D. Sulkin, E. Mojica, and G.L. McKeen	**2076–2079**	Elevated summer temperature effects on megalopal and early juvenile development in the Dungeness crab, *Cancer magister*
Patrick D. Guiney, Philip M. Cook, John M. Casselman, John D. Fitzsimmons, Howard A. Simonin, Erik W. Zabel, and Richard E. Peterson	**2080–2092**	Assessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induced sac fry mortality in lake trout (*Salvelinus namaycush*) from different regions of the Great Lakes
H.B. Blaikie and S.R. Kerr	**2093–2099**	Effect of activity level on apparent heat increment in Atlantic cod, *Gadus morhua*
Lucy A. Dueck and Roy G. Danzmann	**2100–2114**	Matriarchal population structure of introduced rainbow trout (*Oncorhynchus mykiss*) in the Lake Ontario watershed
Jan Horbowy	**2115-2125**	The dynamics of Baltic fish stocks on the basis of a multispecies stock-production model
Marianne Vignaux	**2126–2136**	Analysis of vessel movements and strategies using commercial catch and effort data from the New Zealand hoki fishery
Roman P. Lanno and D. George Dixon	**2137–2146**	Chronic toxicity of waterborne thiocyanate to rainbow trout (*Oncorhynchus mykiss*)
Cosmas Nathanailides	**2147–2155**	Metabolic specialization of muscle during development in cold-water and warmwater fish species exposed to different thermal conditions

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Volume 53, Number 9, September 1996

Volume 53, numéro 9, septembre 1996

	ARTICLES	ARTICLES
C.P. Schneider, R.W. Owens, R.A. Bergstedt, and R. O'Gorman	**1921–1932**	Predation by sea lamprey (*Petromyzon marinus*) on lake trout (*Salvelinus namaycush*) in southern Lake Ontario, 1982–1992
P. Galvin, J. Taggart, A. Ferguson, M. O'Farrell, and T. Cross	**1933–1942**	Population genetics of Atlantic salmon (*Salmo salar*) in the River Shannon system in Ireland: an appraisal using single locus minisatellite (VNTR) probes
Jan Stormer, Frank B. Jensen, and J. Cliff. Rankin	**1943–1950**	Uptake of nitrite, nitrate, and bromide in rainbow trout, *Oncorhynchus mykiss*: effects on ionic balance
Richard D. Brodeur and Matthew T. Wilson	**1951–1963**	Mesoscale acoustic patterns of juvenile walleye pollock (*Theragra chalcogramma*) in the western Gulf of Alaska
Gary R. Fitzhugh, Larry B. Crowder, and James P. Monaghan, Jr.	**1964–1973**	Mechanisms contributing to variable growth in juvenile southern flounder (*Paralichthys lethostigma*)
Brian G. Kotak, Ron W. Zurawell, Ellie E. Prepas, and Charles F.B. Holmes	**1974–1985**	Microcystin-LR concentration in aquatic food web compartments from lakes of varying trophic status
R. Christopher Chambers and Kenneth G. Waiwood	**1986–2003**	Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua*
Barry A. Berejikian, Stephen B. Mathews, and Thomas P. Quinn	**2004–2014**	Effects of hatchery and wild ancestry and rearing environments on the development of agonistic behavior in steelhead trout (*Oncorhynchus mykiss*) fry
R.W. Gauldie	**2015–2026**	Effects of temperature and vaterite replacement on the chemistry of metal ions in the otoliths of *Oncorhynchus tshawytscha*
Øystein Skaala, Knut E. Jørstad, and Reidar Borgstrøm	**2027–2035**	Genetic impact on two wild brown trout (*Salmo trutta*) populations after release of non-indigenous hatchery spawners

Concluded on inside back cover / Suite au verso

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